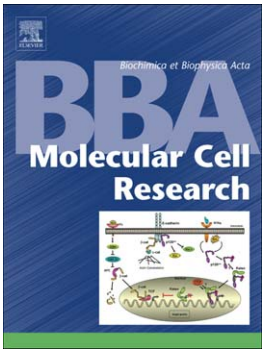

ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca^{2+} signaling

Mart Bittremieux, Jan B. Parys, Paolo Pinton, Geert Bultynck

PII: S0167-4889(16)00003-3
DOI: doi: 10.1016/j.bbamcr.2016.01.002
Reference: BBAMCR 17770

To appear in: *BBA - Molecular Cell Research*

Received date: 21 October 2015
Revised date: 4 January 2016
Accepted date: 5 January 2016

Please cite this article as: Mart Bittremieux, Jan B. Parys, Paolo Pinton, Geert Bultynck, ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca^{2+} signaling, *BBA - Molecular Cell Research* (2016), doi: 10.1016/j.bbamcr.2016.01.002

ER functions of oncogenes and tumor suppressors: modulators of intracellular Ca^{2+} signaling

Mart Bittremieux[1], Jan B. Parys[1], Paolo Pinton[2], Geert Bultynck[1,*]

[1] KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, BE-3000 Leuven, Belgium

[2] University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology and LTTA center, IT-44121 Ferrara, Italy

* Corresponding author. Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, KU Leuven, Campus Gasthuisberg O&N 1 Box 802, Herestraat 49, BE-3000 Leuven, Belgium.
E-mail address: geert.bultynck@med.kuleuven.be
Tel.: +3216330215

Abstract

Intracellular Ca^{2+} signals that arise from the endoplasmic reticulum (ER), the major intracellular Ca^{2+}-storage organelle, impact several mitochondrial functions and dictate cell survival and cell death processes. Furthermore, alterations in Ca^{2+} signaling in cancer cells promote survival and establish a high tolerance towards cell stress and damage, so that the on-going oncogenic stress does not result in the activation of cell death. Over the last years, the mechanisms underlying these oncogenic alterations in Ca^{2+} signaling have started to emerge. An important aspect of this is the identification of several major oncogenes, including Bcl-2, Bcl-XL, Mcl-1, PKB/Akt, and Ras, and tumor suppressors, such as p53, PTEN, PML, BRCA1, and Beclin 1, as direct and critical regulators of Ca^{2+}-transport systems located at the ER membranes, including IP_3 receptors and SERCA Ca^{2+} pumps. In this way, these proteins execute part of their function by controlling the ER-mitochondrial Ca^{2+} fluxes, favoring either survival (oncogenes) or cell death (tumor suppressors). Oncogenic mutations, gene deletions or amplifications alter the expression and/or function of these proteins, thereby changing the delicate balance between oncogenes and tumor suppressors, impacting oncogenesis and favoring malignant cell function and behavior. In this review, we provided an integrated overview of the impact of the major oncogenes and tumor suppressors, often altered in cancer cells, on Ca^{2+} signaling from the ER Ca^{2+} stores.

Keywords: tumor suppressors, oncogenes, calcium, cancer, cell death, endoplasmic reticulum

List of abbreviations

a.a.: amino acid
ATP: adenosine triphosphate
BH domain: Bcl-2-homology domain
BI-1: Bax inhibitor-1
BIRD-2: Bcl-2/IP_3R disruptor-2
BRCA1: breast and ovarian cancer susceptibility gene 1
$[Ca^{2+}]_{cyt}$: cytosolic Ca^{2+} concentration
CLL: chronic lymphocytic leukemia
DL-BCL: diffuse large B-cell lymphoma
ER: endoplasmic reticulum
GAP: GTPase-activating protein
IICR: IP_3-induced Ca^{2+} release
IP_3R: inositol 1,4,5-trisphosphate receptor
MAM: mitochondria-associated ER membrane
MCU: mitochondrial calcium uniporter
MEF: mouse embryonic fibroblast
MOMP: mitochondrial outer membrane permeabilization
mPTP: mitochondrial permeability transition pore
mTOR: mammalian target of rapamycin
OMM: outer mitochondrial membrane

PDK1: phosphatidylinositol-dependent kinase 1
PDT: photodynamic therapy
PEST region: proline/glutamic acid/serine/threonine-containing region
PI3K: phosphoinositide 3-kinase
PIP_2: phosphatidylinositol 4,5-bisphosphate
PIP_3: phosphatidylinositol 3,4,5-trisphosphate
PKB: protein kinase B
PKC: protein kinase C
PLC: phospholipase C
PML: promyelocytic leukemia protein
PP2A: protein phosphatase 2A
PTEN: phosphatase and tensin homolog deleted on chromosome 10
RA2: Ras-associating domain 2
ROS: reactive oxygen species
RyR: Ryanodine receptor
SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase
tBid: truncated Bid
TCR: T-cell receptor
TKO DT40 cells: triple-IP_3R-knockout DT40 cells
VDAC1: voltage-dependent anion channel 1

Introduction

Intracellular Ca^{2+} signals regulate cell function and cell survival by controlling many processes. Ca^{2+} pumps, Ca^{2+} channels, Ca^{2+} exchangers and Ca^{2+}-binding proteins present at the plasma membrane and in different cellular compartments tightly control the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) [1–3]. In resting condition, the $[Ca^{2+}]_{cyt}$ is maintained at a concentration of approximately 100 nM, whereas the extracellular $[Ca^{2+}]$ is about 1 mM [1,4]. In response to various stimuli, the intracellular $[Ca^{2+}]$ can increase to the micromolar range in the cytosol [1–3] and reach 10 μM and higher in microdomains like the interface between the endoplasmic reticulum (ER) and the mitochondria [5,6]. This $[Ca^{2+}]$ increase can be evoked by mobilizing Ca^{2+} from intracellular stores, such as ER and Golgi apparatus, or by Ca^{2+} entry from the extracellular environment [1–4]. The free $[Ca^{2+}]$ in the ER, the main intracellular Ca^{2+} store, varies around 500 μM [7]. Although the free $[Ca^{2+}]$ is around 500 μM, the total $[Ca^{2+}]$ in the ER is more than 2 mM as a result of the action of different Ca^{2+} buffering proteins such as calreticulin, Grp94 and BiP. These proteins bind Ca^{2+} with low affinity and high capacity [7]. The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), localized in the ER membrane, lowers the $[Ca^{2+}]_{cyt}$ by pumping Ca^{2+} from the cytosol into the ER in an adenosine triphosphate (ATP)-dependent manner [8]. On the other hand, inositol 1,4,5-trisphosphate receptors (IP_3Rs) release Ca^{2+} from the ER store, allowing it to function as an intracellular messenger in several signal transduction pathways [9]. The IP_3R, of which three isoforms exist (IP_3R1, IP_3R2, and IP_3R3), is activated by elevated cytosolic levels of IP_3, produced by phospholipase C (PLC) after stimulation of G-protein-coupled receptors or receptor tyrosine kinases by hormones, growth factors or antibodies [9–11]. IP_3R activity is regulated by Ca^{2+} itself as well. Depending on the intracellular $[Ca^{2+}]$, Ca^{2+} either activates (low $[Ca^{2+}]_{cyt}$) or inhibits (high $[Ca^{2+}]_{cyt}$) the channel [12–14]. Furthermore, regulatory proteins [9,10,15], protein kinases and phosphatases [16] and ATP [17–20] also modulate the Ca^{2+}-flux properties of the IP_3R. Ca^{2+} released from the ER can be efficiently accumulated by the mitochondria, thereby directly impacting several mitochondrial functions [21–23]. Ca^{2+} is transferred across the outer mitochondrial membrane (OMM) by voltage-dependent anion channel 1 (VDAC1), a weakly anion-selective channel that is permeable to Ca^{2+} as well [24,25]. Subsequently, the mitochondrial calcium uniporter (MCU) complex removes Ca^{2+} from the mitochondrial intermembrane space and imports Ca^{2+} into the matrix, a process that is driven by the negative mitochondrial membrane potential generated by electron transport [26]. In the mitochondria-associated ER membranes (MAMs), which are mitochondria-associated ER subfractions involved in multiple cellular processes, including lipid synthesis, ER stress, autophagy and apoptosis, ER-mitochondrial Ca^{2+} transport is facilitated by coupling of the IP_3R to VDAC1 via glucose regulated protein 75 [27,28].

Intracellular Ca^{2+} signaling impacts many cellular processes, including mitochondrial bioenergetics, senescence, mitophagy, autophagy, and apoptosis [29–33]. First, mitochondrial bioenergetics are maintained by constitutive low-level IP_3R-mediated Ca^{2+} transfer from the ER to the mitochondria [29]. Ca^{2+} stimulates mitochondrial respiration and ATP production by promoting the activities of the Ca^{2+}-dependent rate-limiting enzymes of the tricarboxylic acid cycle, i.e. alpha-ketoglutarate, isocitrate and pyruvate dehydrogenases [34]. Importantly, absence of constitutive low-level ER-mitochondrial Ca^{2+} signaling results in inhibition of the aforementioned dehydrogenases and can lead to activation of AMP-activated protein kinase, stimulating pro-survival mammalian target of rapamycin (mTOR)-independent autophagy [29,32]. Second, apoptosis is another process depending on intracellular Ca^{2+} signaling. In cells suffering from varying forms of stress or damage, excessive

Ca^{2+} is released from the ER through the IP_3R and transferred to the mitochondria via VDAC1 and MCU, causing mitochondrial Ca^{2+} overload [35]. This in turn leads to opening of the mitochondrial permeability transition pore (mPTP) [36], loss of mitochondrial membrane potential, permeabilization and rupture of the OMM and subsequent release of mitochondrial pro-apoptotic factors from the intermembrane space into the cytosol, such as cytochrome c, apoptosis-inducing factor and endonuclease G. Recently, the disassembly of respiratory chain complex II due to loss of cardiolipin was proposed to be responsible for cell death triggered in response to mitochondrial Ca^{2+} overload [37]. Third, intracellular Ca^{2+} is also an important regulator of macro-autophagy, a lysosomal degradation process disposing long-lived proteins, protein aggregates, damaged organelles and intracellular pathogens [32,38–41]. In this way, macro-autophagy, which will be further referred to as autophagy, maintains cellular homeostasis and promotes cell survival during stress conditions, though cell death is induced if the stress is too high or persists for a longer time [38,42]. Depending on the cellular state, intracellular Ca^{2+} signaling either inhibits or stimulates autophagy [42]. In healthy cells, basal autophagy is suppressed by small, spontaneous IP_3R-mediated Ca^{2+} signals transferred from the ER into the mitochondria that drive ATP production [29,32]. In contrast, Ca^{2+} signaling is enhanced in stressed cells, leading to elevated $[Ca^{2+}]_{cyt}$ that promotes autophagy by activating autophagy-stimulating proteins, such as Ca^{2+}/Calmodulin-dependent protein kinase kinase-β [42,43]. Besides the control of macro-autophagy by intracellular Ca^{2+} signaling, also mitophagy, a process by which excessive or damaged mitochondria are removed, is modulated by Ca^{2+} uptake into the mitochondria: mitochondrial structure, membrane potential and reactive oxygen species (ROS) are not only important regulators of the Ca^{2+} uptake capacity of the mitochondria, but also determine whether or not to trigger mitophagy [31]. Finally, intracellular Ca^{2+} signaling plays an important role in cellular senescence, a protective mechanism against oncogenic events and tumorigenesis [30]. Loss of retinoblastoma and p53 pathways are usually involved in senescence, but recently it was revealed that the IP_3R2 and MCU regulate this process as well [30]. Cells escaped oncogene-induced and replicative senescence by loss of IP_3R2 as well as loss of MCU, since IP_3R2-mediated Ca^{2+} release and subsequent mitochondrial Ca^{2+} accumulation through MCU lead to a decreased mitochondrial membrane potential, followed by ROS production and accumulation and eventually senescence.

During the last years, it has become clear that the remodeling of intracellular Ca^{2+} signaling pathways is a hall-mark of cancer cells that favors their survival and augments their cell death resistance [44–47]. Many tumor cells display altered expression levels of proteins, including oncogenes and tumor suppressors that have functions at the MAMs and directly impact ER-mitochondrial Ca^{2+} transfer (Fig. 1) [48]. Oncogenes promote cell survival by suppressing pro-apoptotic ER-mitochondrial Ca^{2+} signaling events, whereas tumor suppressors stimulate these ER-mitochondrial Ca^{2+} fluxes. Hence, it is not surprising that toxic Ca^{2+} signaling events are circumvented in cancer cells by the upregulation of oncogenes and/or the downregulation of tumor suppressors. The Ca^{2+}-mediated cell death response is modulated either directly or indirectly in cancer cells. Cell death is escaped indirectly in tumor cells by a lowered ER Ca^{2+} content which prevents toxic, pro-apoptotic Ca^{2+} signals emanating from the ER and avoids mitochondrial Ca^{2+} overload (Fig. 2) [46,47]. For example, the oncogenes Mcl-1, Bcl-2 and Bcl-XL lower the ER store content by stimulating IP_3Rs outside of the MAMs, thereby increasing Ca^{2+} leak from the ER. Oncogenes Bcl-2 and Ras also decrease the ER Ca^{2+} levels by inhibiting the SERCA pump or by lowering SERCA2b expression levels, respectively. On the other hand, p53, which is frequently mutated in tumor cells, increases the ER store content by stimulating

SERCA activity. Apoptotic cell death can also be circumvented in cancer cells by a direct modulation of toxic ER-mitochondrial Ca^{2+} signaling events (Fig. 3) [46,48–50]. This occurs through an upregulation of oncogenes that inhibit excessive, toxic Ca^{2+} signals, including Bcl-2 and PKB/Akt, which both inhibit the IP_3R, and Bcl-XL, which inhibits VDAC1. In contrast, tumor suppressors that promote pro-apoptotic Ca^{2+} signaling events are downregulated or mutated in cancer cells. This includes PTEN and BRCA1, which stimulate IP_3Rs in the MAMs. The direct and indirect modulation of pro-apoptotic Ca^{2+} transfers from the ER to the mitochondria by oncogenes and tumor suppressors will be discussed in detail in the paragraphs below. Furthermore, oncogenes that sensitize IP_3Rs to low levels of IP_3, such as Bcl-XL and Mcl-1, enhance pro-survival Ca^{2+} oscillations and stimulate mitochondrial bioenergetics by enhancing ATP production, hence preserving cell proliferation and cell survival (Fig. 4) [46]. The present review aims to offer a thorough discussion of the various oncogenes and tumor suppressors that are most often altered in cancer cells and influence Ca^{2+} signaling from the ER.

Bcl-2

Bcl-2 is a proto-oncogene localized at the mitochondria and the ER that serves as an anti-apoptotic protein by preventing mitochondrial outer membrane permeabilization (MOMP) [51–53]. Bcl-2 is the founding member of the Bcl-2-protein family, which consists of different anti-apoptotic and pro-apoptotic members [54,55]. At the mitochondria, Bcl-2 scaffolds and neutralizes pro-apoptotic proteins like Bax/Bak and activator BH3-only proteins such as Bim and truncated Bid (tBid), thereby hindering the activation and oligomerization of Bax/Bak [51,52]. Bcl-2's anti-apoptotic function is counteracted by sensitizer BH3-only proteins, like Bad, Puma and Noxa. At the ER, Bcl-2 prevents excessive Ca^{2+} signaling that would lead to mitochondrial Ca^{2+} overload and opening of the mPTP [56]. At the structural level, the anti-apoptotic Bcl-2 protein is organized in different Bcl-2-homology (BH) domains, including the BH4, BH3, BH1 and BH2 domain, and a C-terminal transmembrane domain for anchoring in membranes of intracellular organelles, including the mitochondria and the ER [57]. The BH3-BH1-BH2 domains together form a hydrophobic cleft, which targets and scaffolds the BH3 domain of pro-apoptotic Bcl-2-family members, such as the multidomain proteins Bax/Bak and the BH3-only proteins Bim, Bid and Bad. Bcl-2 expression is dysregulated in a variety of cancers, including chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DL-BCL) and non-small cell lung cell carcinoma [58–60]. In these cancers, the Bcl-2-expression levels are upregulated. Different mechanisms for Bcl-2 upregulation in cancers have been identified, including (i) a chromosomal translocation t(14;18) [58,61], which places Bcl-2 under the control of an IgG promoter sequence, (ii) downregulation of endogenous miRNA targeting Bcl-2 [62], and (iii) hypomethylation of the Bcl-2 gene resulting in an altered epigenetic regulation of Bcl-2 [63].

The impact of anti-apoptotic Bcl-2 on the ER Ca^{2+} homeostasis and dynamics is complex. Different but not mutually exclusive models have been proposed for ER-localized Bcl-2. Earlier work showed that anti-apoptotic Bcl-2 lowered the steady-state ER Ca^{2+}-store content (Fig. 2) [64–66], thereby protecting the mitochondria from Ca^{2+} overload. Several underlying mechanisms were proposed, including Bcl-2 as a Ca^{2+}-leak channel [67], Bcl-2 as an IP_3R sensitizer [68,69] and Bcl-2 as a SERCA regulator [70]. (i) The original proposal that Bcl-2 could function as a putative Ca^{2+}-pore-forming protein [67] has been excluded by a study showing that the effects of Bcl-2 in Ca^{2+} signaling were not

dependent on its putative pore-forming domain [71]. (ii) Bcl-2's function as an IP_3R sensitizer was supported by evidence from Bax/Bak-knockout mouse embryonic fibroblasts (MEF cells), which display an increased ratio of Bcl-2 over Bax/Bak [68]. Bax/Bak-knockout MEF cells displayed a decreased ER Ca^{2+}-store content that was due to an increased sensitivity of IP_3Rs towards its agonist IP_3. As such, in the presence of excess Bcl-2, hypersensitive IP_3Rs could become activated at basal IP_3 levels, thereby promoting the basal Ca^{2+} leak from the ER through open IP_3R channels and lowering the steady state ER Ca^{2+} levels. At the molecular level, IP_3R sensitization was due to an increased protein kinase A-dependent phosphorylation on Ser1755 [68]. Follow-up work implicated an even more complex regulation of the Bcl-2-dependent IP_3R phosphorylation status, involving a role for calcineurin and protein phosphatase 1/Inhibitor-1 [72–74]. Also, the effect of Bcl-2 on ER Ca^{2+}-store content appears to be dynamically regulated by phosphorylation. Bcl-2 phosphorylation mediated by c-Jun N-terminal protein kinase, neutralizing its anti-apoptotic properties, counteracts Bcl-2's ability to lower ER Ca^{2+} levels [75]. Another mechanism for the IP_3R sensitization by Bcl-2 has also been proposed. Bcl-2 was found to directly bind the C-terminal part of the IP_3R channel, i.e. a domain that is in close proximity to the Ca^{2+}-channel pore domain (Fig. 5) [69]. Bcl-2 targeted the 6^{th} transmembrane domain of the IP_3R, thereby sensitizing the channel and promoting pro-survival Ca^{2+} oscillations linked to enhanced mitochondrial bioenergetics (Fig. 4). Hence, the C-terminal site in IP_3R responsible for Bcl-2 binding corresponded to the site previously identified for Bcl-XL [76,77]. The affinity of the binding of Bcl-2 to the C-terminal IP_3R domain has not been determined. Bcl-2 overexpression in DT40 cells caused a lowering of the ER Ca^{2+} levels in an IP_3R-dependent manner [69]. Consistent with this, Bcl-2 overexpression was more effective in protecting IP_3R-expressing than IP_3R-deficient DT40 cells against pro-apoptotic stimuli. (iii) Bcl-2 has been shown to directly interact with various SERCA isoforms (SERCA1 and SERCA2b), thereby limiting their ER Ca^{2+}-uptake activity (Fig. 2) [70,78]. The binding of Bcl-2 to SERCA has also been proposed to destabilize the SERCA protein, thereby lowering its protein levels.

Besides its effect on the ER Ca^{2+}-store content, Bcl-2 can also directly control ER Ca^{2+}-release mechanisms without impacting the ER Ca^{2+}-store content [79–81]. Bcl-2 directly interacts with the three isoforms of the IP_3R channel, thereby inhibiting their function (Fig. 3). Bcl-2 overexpression in immature T cells expressing low Bcl-2 levels suppressed IP_3R-mediated Ca^{2+} release elicited by strong T-cell receptor (TCR) stimulation or by a cell-permeable version of IP_3 [80,82]. Remarkably, Bcl-2 did not suppress Ca^{2+} signals triggered by weak TCR stimulation. This suggests that Bcl-2 mainly suppresses excessive, pro-apoptotic Ca^{2+} signaling events but not pro-survival Ca^{2+} signaling events like Ca^{2+} oscillations. The frequency of these Ca^{2+} oscillations even appeared to be stimulated, supporting a putative sensitizing effect of Bcl-2 on IP_3Rs under certain circumstances. In addition, Bcl-2 directly regulated the channel properties of the IP_3R, since purified Bcl-2 could suppress IP_3R single-channel activity and could interact with purified IP_3R domains [80]. The Bcl-2 domain responsible for IP_3R interaction and inhibition was the N-terminal BH4 domain [83]. This domain was essential and sufficient for binding IP_3Rs, suppressing IP_3R-mediated Ca^{2+}-flux properties and protecting against apoptotic stimuli. The binding of the central IP_3R domain to BH4-Bcl-2 occurred with an apparent affinity in the low μM range, while the IC_{50} for the inhibition of IP_3-induced Ca^{2+} release by BH4-Bcl-2 in permeabilized cell systems was about 30 μM [83,84]. The alpha-helical properties of the BH4 domain and the presence of Lys17 in the center of this domain were critical for IP_3R interaction and regulation [84,85]. Bcl-2 binding to IP_3R was mapped to a region of 20 amino acids (a.a. 1389-1408 in the mouse IP_3R1), located in the central, modulatory domain of the channel (Fig. 5) [86]. A peptide

corresponding to this Bcl-2-binding site on IP_3Rs was sufficient to disrupt IP_3R/Bcl-2-complex formation [86], thereby augmenting TCR-triggered IP_3R-mediated Ca^{2+} release and apoptosis without being cytotoxic by itself [86]. A modified version of this peptide coupled to TAT and stabilized (called BIRD-2 for Bcl-2/IP_3 receptor disruptor-2) triggered spontaneous, large Ca^{2+}-signaling events that resulted in apoptotic cell death in CLL cells [87]. Similar results were obtained in DL-BCL cells, in which the sensitivity of the cancer cells correlated with the expression level of the IP_3R2, the IP_3R isoform with the highest sensitivity towards its ligand IP_3 [88]. Furthermore, DL-BCL cells that were less sensitive to BH3 mimetics were more sensitive to BIRD-2 and *vice versa*, suggesting a dual role for Bcl-2 in B-cell cancers at both the mitochondria and the ER [49]. Not only CLL and DL-BCL cells but also multiple myeloma, follicular lymphoma and small cell lung cancer cells appeared to be dependent on the BH4-domain biology of Bcl-2 and thus were sensitive to BIRD-2 treatment [89,90]. Moreover, *in vivo* injection of BIRD-2 suppressed the *in vivo* growth of multiple myeloma cancer cells xenografted on immunodeficient mice [89]. Prolonged exposure of multiple myeloma cancer cells to BIRD-2 resulted in an increase in Bim levels, thereby sensitizing cancer cells towards the BH3-mimetic drugs ABT-263 and ABT-199 [89]. This was further supported by evidence in small cell lung cancer cells, in which ABT-263 potentiated BIRD-2-induced cell death [90]. Moreover, BIRD-2-induced cell death could be potentiated by other Bcl-2 inhibitors that also impact Ca^{2+}-transport systems like HA14-1 [91]. BIRD-2 can trigger Ca^{2+}-induced apoptosis through both caspase-dependent and caspase-independent mechanisms, since caspase inhibitors suppressed BIRD-2-induced cell death in Bcl-2-positive lymphoid malignancies [89] but not in small cell lung cancer cells [90]. In the latter, BIRD-2-induced apoptosis was counteracted by calpain inhibitors.

The "dual" role of Bcl-2 as a dampener of the ER Ca^{2+}-store content or as a direct inhibitor of the IP_3R not affecting the ER Ca^{2+}-store content has been causing controversy in the field [92]. Yet, the varying effect of Bcl-2 on ER Ca^{2+} could be explained by different factors. First, the reduced ER Ca^{2+} levels upon Bcl-2 overexpression might be mediated by a downstream ER Ca^{2+}-leak pathway that is activated by Bcl-2. For instance, Bcl-2 interacts with Bax Inhibitor-1 (BI-1), an ER-located Ca^{2+}-leak channel belonging to the transmembrane Bax Inhibitor-1 motif-containing protein family [93–96]. Interestingly, Bcl-2 overexpression only lowered the steady state ER Ca^{2+} levels in BI-1-expressing cells but not in BI-1-knockout cells [97], indicating that Bcl-2 may lower the ER Ca^{2+}-store content in a BI-1-dependent manner. Second, the effect of Bcl-2 on ER Ca^{2+} might be cell type dependent. For instance, high expression levels of IP_3R3 in certain cell types might favor Bcl-2's ability to lower ER Ca^{2+} levels as shown in the DT40 triple-IP_3R knockout cell system in which Bcl-2 only lowered the ER Ca^{2+} content in IP_3R3-expressing cells. Third, the cellular effects of Bcl-2 have also been shown to depend on its localization and its concentration. For instance, transient overexpression of mitochondrially-targeted Bcl-2 or wild-type Bcl-2, but not ER-targeted Bcl-2, could actually induce apoptosis in certain cells [98]. Furthermore, this effect might be dependent on its cellular concentration, since excessive overexpression has been implicated to trigger apoptosis [99]. Hence, the lowering of the ER Ca^{2+}-store content in stably Bcl-2-overexpressing cells might be a compensatory mechanism.

Besides IP_3Rs, Bcl-2 proteins also target and inhibit ryanodine receptors (RyRs) [100], another class of intracellular Ca^{2+}-release channels. All RyR isoforms (RyR1, RyR2 and RyR3) interacted with Bcl-2. The binding of Bcl-2 to RyRs occurred via its central domain, consistent with the fact that the stretch of amino acids responsible for Bcl-2 binding to the IP_3R binding is highly conserved in RyRs [101]. The molecular determinants in Bcl-2 responsible for RyR binding were similar but not identical to the

ones responsible for IP_3R binding [100]. While the BH4 domain of Bcl-2 was responsible for RyR binding, the Lys17 residue of Bcl-2 appeared dispensable for the interaction with RyRs. Also, the BH4 domain of Bcl-2 was sufficient for inhibiting RyRs, overexpressed in HEK cells or endogenously present in rat hippocampal neurons. The binding of Bcl-2 to RyRs also appeared independent of its hydrophobic cleft, which is responsible for binding the BH3 domain of pro-apoptotic Bcl-2-family members [102]. The relevance of the RyR/Bcl-2 interaction for cancer cell survival ought to be further studied. Yet, recently, elevated RyR3 expression has been implicated in breast cancer risk [103]. Knocking down RyR3 in breast cancer cells impaired cell proliferation and migration. Furthermore, certain breast cancers host a signal nucleotide polymorphism in the 3'-UTR of the RyR3 gene, which prevented binding of a regulatory miRNA and caused elevated RyR3 expression in patient samples. Patients carrying the signal nucleotide polymorphism had a higher risk of developing breast cancer and its occurrence correlated to increased micro-calcification and poor progression-free survival. Also in breast cancer cells, a strong RyR2 upregulation has been described upon epithelial-mesenchymal transition, a process that promotes invasive properties of cells and that is stimulated by hypoxia or epidermal growth factor [104]. However, the relevance of RyR/Bcl-2 complexes in breast cancer properties and their ability to undergo epithelial-mesenchymal transition remains to be further studied.

Bcl-XL

Bcl-XL is an anti-apoptotic protein related to Bcl-2. This proto-oncogene is expressed in many tissues [105]. At the mitochondria, Bcl-XL scaffolds and neutralizes pro-apoptotic Bcl-2-family members like Bak/Bax and BH3-only proteins Bim and Bid, thereby preventing MOMP and promoting cell survival [51]. Bcl-XL is frequently overexpressed in cancers by which apoptosis is inhibited and cell survival is promoted. High levels of Bcl-XL have been detected in advanced and relapsed multiple myeloma, prostate, rectal, small-cell lung, gastric, pancreatic cancer, and tongue carcinomas [106,107]. Furthermore, constitutive activation of the epidermal growth factor receptor in human glioblastoma cells increases the expression of Bcl-XL, resulting in apoptosis resistance [108]. Of interest, thrombocytopenia is a major side effect of inhibiting Bcl-XL in anti-cancer therapy, since Bcl-XL is essential to maintain platelet survival by restricting Bax activity [109]. Nevertheless, selective Bcl-XL inhibitors have been reported to be highly efficacious in combination with docetaxel to target a variety of solid tumors [110].

Bcl-XL can directly bind the IP_3R channel and regulate its single-channel Ca^{2+} flux properties [76]. Bcl-XL caused a potent sensitization of the IP_3R, promoting its opening in response to very low [IP_3] (10 nM). At the molecular level, Bcl-XL bound all three IP_3R isoforms by targeting a site located outside the first 600 amino acids of the IP_3R1. Further experiments revealed that the C-terminal part of the IP_3R between a.a. 2512 and 2750 contained a Bcl-XL-binding site (Fig. 5) [76]. Bcl-XL overexpression promoted IP_3R-dependent Ca^{2+} oscillations. Interestingly, purified tBid and Bax prevented Bcl-XL binding to IP_3Rs and the sensitization of IP_3R channels by Bcl-XL, suggesting an involvement of Bcl-XL's hydrophobic cleft in the regulation of IP_3Rs. This was underpinned by follow-up work, indicating that the C-terminal site of the IP_3R contains two domains reminiscent of BH3 domains, which may recruit Bcl-2/Bcl-XL via their hydrophobic cleft [111]. In DT40 cells, the decrease of steady-state ER Ca^{2+} levels and protection against strong B-cell receptor stimulation by overexpression of Bcl-XL

required the presence of IP_3Rs (Fig. 2) [76]. Consistent with this, Bcl-XL promoted spontaneous Ca^{2+} oscillations in wild-type but not in triple-IP_3R-knockout (TKO) DT40 cells. As a consequence, Bcl-XL boosted mitochondrial bioenergetics in an IP_3R-dependent manner, increasing nicotinamide adenine dinucleotide phosphate production (Fig. 4). Further work revealed that all three IP_3R isoforms could enhance Bcl-XL's anti-apoptotic properties [77], consistent with the fact that Bcl-XL can bind to all three IP_3R isoforms [76] and can promote Ca^{2+} oscillations mediated by IP_3R1, IP_3R2 or IP_3R3 [77]. However, Bcl-XL only lowered the ER Ca^{2+}-store content of IP_3R3-, but neither of IP_3R1- or IP_3R2-expressing TKO-DT40 cells. This suggested that lowering of the ER Ca^{2+}-store content was not essential for the anti-apoptotic properties of Bcl-XL. Of note, similarly to Bcl-2, Bcl-XL too could directly bind to RyRs, which involved its BH4 domain and a Lys residue located in the BH3 domain [112]. Bcl-XL binding to RyRs suppressed Ca^{2+} release through the channels. The presence of these complexes in cancer cells and their relevance for oncogenic properties remain unknown.

Besides IP_3Rs and RyRs, Bcl-XL has been implicated in targeting Ca^{2+}-flux pathways of the mitochondria like VDAC1, although different outcomes have been reported with Bcl-XL inhibiting or stimulating VDAC1-mediated Ca^{2+} uptake into the mitochondria [113–115]. The inhibitory effect of Bcl-XL on VDAC1 could be attributed to its BH4 domain [116]. Of course, these effects of Bcl-XL on the rate of mitochondrial Ca^{2+} uptake could indirectly impact the ER Ca^{2+}-release properties. For instance, decreased mitochondrial Ca^{2+} uptake, e.g. by Bcl-XL inhibiting VDAC1, may lead to an increase in cytosolic Ca^{2+} signaling for a given Ca^{2+} release from the ER. Furthermore, the inhibitory action of Bcl-XL on VDAC1 may be an additional mechanism to prevent toxic mitochondrial Ca^{2+} overload (Fig. 3). In contrast to these studies, Bcl-XL has also been reported to enhance VDAC1-mediated mitochondrial Ca^{2+} uptake [114]. Although we and others [113,115,116] reported an inhibitory role for Bcl-XL on VDAC1, these data could reflect the dual role of VDAC1 in both mediating survival and apoptosis signaling [117]. As such, Bcl-XL may promote the transfer of pro-survival Ca^{2+} signaling while inhibiting pro-apoptotic Ca^{2+} signaling. Alternatively, differences in the experimental methods could account for this. For instance, many experiments performed by White *et al.* were based on mitochondrial Ca^{2+} uptake experiments in permeabilized cells where all VDAC1 channels across the mitochondrial outer membrane will participate in mitochondrial Ca^{2+} uptake [114]. In contrast, experiments in other studies relied on mitochondrial Ca^{2+} uptake measurements in intact cells exposed to agonists for which Ca^{2+} would be preferentially transferred via ER-mitochondrial contact sites [113,115,116]. Nevertheless, since a stimulatory effect of VDAC1 by Bcl-XL has never been shown in direct measurements based on purified VDAC1 channels, the molecular properties and the relevance of the VDAC1/Bcl-XL connection in promoting mitochondrial Ca^{2+} transfer ought to be further scrutinized.

Mcl-1

Mcl-1 is an anti-apoptotic member of the Bcl-2-protein family and has many important cellular functions. This proto-oncogene is crucial for the development and maintenance of several cell types including lymphocytes [118,119], neurons [120] and hematopoietic stem cells [121]. Additionally, Mcl-1 plays an important role in early embryogenesis [122]. The Mcl-1 protein has a very short half-life and is structurally different from the other anti-apoptotic Bcl-2-family members [123]. The sequence of the putative BH4 domain of Mcl-1 substantially differs from the sequences of the BH4

domain of the Bcl-2 and Bcl-XL proteins. Furthermore, its N-terminus contains two PEST (proline/glutamic acid/serine/threonine-containing) regions, which affect the rate of turnover and contain several sites for phosphorylation [123]. Mcl-1's pro-survival functions are advantageous to many types of cancer. Overexpression of Mcl-1 has been observed in hematopoietic and in lymphoid cancers as well as in solid tumors [123]. This is supported by an analysis of somatic copy-number alterations in different cancer types, showing that the gene encoding for Mcl-1 is frequently amplified in a broad spectrum of cancer cells [124]. Mcl-1 overexpression induces resistance to chemotherapeutic agents like paclitaxel and vincristine, but also to Bcl-2 inhibitors such as ABT-737, while the sensitivity of chemoresistant cells to these compounds can be restored after Mcl-1 silencing [123,125].

One study reported the binding of Mcl-1 to IP_3Rs [69]. It was shown that besides Bcl-2 and Bcl-XL, Mcl-1 too targeted the C-terminal tail of the IP_3R (Fig. 5). All three Bcl-2-family members bound the IP_3R domain with similar affinities. It was proposed that the last transmembrane domain of the IP_3R was essential for these interactions. Mcl-1 enhanced IP_3R-mediated Ca^{2+} release, resulting in decreased steady-state ER Ca^{2+} levels (Fig. 2) [69]. Store depletion in Mcl-1-expressing cells became more prevalent in the presence of low [IP_3], indicating that the sensitivity of IP_3-dependent Ca^{2+} release is enhanced by Mcl-1. The Mcl-1-mediated IP_3R sensitization also contributes to low-level IP_3R-mediated Ca^{2+} signaling from the ER to the mitochondria and therefore stimulates mitochondrial bioenergetics (Fig. 4). Also, Mcl-1's anti-apoptotic properties were promoted when IP_3Rs were present in the DT40 cells [69]. Yet, the protective effect of Mcl-1 overexpression in IP_3R-expressing DT40 cells was less pronounced than the effects observed for Bcl-2 or Bcl-XL overexpression.

Of interest, Mcl-1 has recently been implicated to bind with high affinity to VDAC1, thereby stimulating VDAC1's Ca^{2+}-flux properties and thus increasing mitochondrial Ca^{2+} uptake, hence promoting ATP production (Fig. 4) [126]. Among all Bcl-2-family members, Mcl-1 was the strongest VDAC1-binding protein. The VDAC1/Mcl-1 complex appeared to be important for the survival of cancer cells, since the increase in mitochondrial [Ca^{2+}] stimulates ROS production which in turn promotes cell migration [126]. In addition to this, Mcl-1 has recently been implicated in the control of mitochondrial dynamics by promoting Drp1-mediated mitochondrial fission and preventing mitochondrial hyperpolarization, which limits mitochondrial Ca^{2+} uptake [127].

Bok

Bok, Bcl-2-related ovarian killer, is a multi-BH domain-containing pro-apoptotic protein that resembles Bax/Bak. It is scaffolded by Mcl-1 and Bfl-1, but not by Bcl-2 or Bcl-XL [128]. Although a first report suggested that Bok expression was restricted to reproductive tissues [128], Bok appears to be expressed in many tissues besides ovary with high levels in brain, lung, spleen and stomach [129]. Since Bok-knockout mice develop normally, it was proposed that the functions of Bok largely overlap with Bax/Bak or are only critical in certain conditions of cell stress [129]. Compared to Bax/Bak, the role of Bok in apoptosis is however much less characterized. A major portion of the endogenous Bok protein resides outside the mitochondria, including the ER, Golgi and nucleus [130]. Bok overexpression in cells resulted in apoptosis that required the presence of Bax/Bak proteins [130]. Bok overexpression caused a rapid disintegration and fragmentation of the ER and Golgi independently of downstream caspase 3 activation and apoptosis. ER/Golgi-targeting of Bok was

dependent on its C-terminal transmembrane domain. Cells lacking Bok displayed a normal sensitivity towards cell death stimuli like staurosporine and etoposide but displayed an increased sensitivity and abnormal ER-stress response to brefeldin A, a pro-apoptotic ER-stress inducer that disrupts ER-Golgi trafficking [130,131]. In further work, it was shown that Bok has an important function in mediating cell death upstream of Bax/Bak in response to ER stress, since Bok-deficient cells were protected against thapsigargin- or bortezomib-induced apoptosis [132]. The presence of Bok was essential for the activation of Bim, activating transcription factor 4 and CCAAT/enhancer-binding protein homologous protein, pro-apoptotic components of the unfolded protein response. The role of Bok in cancer is not fully understood, but deletions in the Bok gene were identified in high-resolution analyses of somatic copy-number alterations from more than 3000 cancer specimens as a frequent event to occur in different types of cancer [124]. However, in contrast to Bax deletion, which accelerated cancer development, Bok deletion did not accelerate the development of lymphomas in Eμ-*Myc* transgenic mice [129].

Interestingly, among all Bcl-2-family members, Bok displayed the strongest binding to the IP_3R [133]. Bok binding to IP_3Rs was isoform-specific, since IP_3R1 and IP_3R2, but not IP_3R3, were targets for Bok. IP_3Rs may serve as a sink for Bok, as most of the cellular Bok was found in complex with IP_3Rs. The domains responsible for IP_3R1/Bok interaction were on the one hand a stretch of amino acids (PSRKKAKEP) located between a.a. 1895 and 1903 of IP_3R1 (Fig. 5) and on the other hand a stretch of amino acids (LGREYV) located between a.a. 34 and 39, corresponding to a part of the N-terminal BH4 domain of Bok. Furthermore, Bok-deficient cells displayed increased levels of IP_3R1 and decreased IP_3R2 and IP_3R3 levels [133]. Yet, overall, Bok-expressing and Bok-deficient cells displayed similar IP_3-induced Ca^{2+} release (IICR) properties. Bok also did not cause major alterations in IP_3R downregulation by the ubiquitin proteasome pathway and Bok was co-degraded with IP_3R by this system in conditions of persistent activation of the IP_3-dependent signaling pathway [133]. However, the association of Bok with the IP_3R protected these channels from proteolytic digestion by chymotrypsin, which has a cleavage site in the IP_3R in the proximity of the Bok-binding site [133]. Bok also protected IP_3Rs against cleavage by caspase 3, an apoptosis executioner caspase, since Bok-deficient cells exposed to apoptotic stimuli displayed a rapid cleavage of IP_3Rs into an N-terminal 170-kDa fragment and a C-terminal 95-kDa fragment containing the channel pore. This indicates that the Bok-expression levels may critically control the caspase 3-dependent cleavage of IP_3Rs. This is particularly relevant in the context of the conflicting results on whether or not IP_3Rs are *bona fide* substrates of caspase 3. About 15 years ago, the team of Mikoshiba reported that IP_3R1 was a caspase 3 substrate as IP_3R1 cleavage upon apoptotic stimuli was only observed in cells expressing caspase 3 [134]. Accordingly, a caspase 3-cleavage site (DEVD) was identified in IP_3R1 (a.a. 1888-1892 in mouse IP_3R1) (Fig. 5). The removal of this site (by mutation) diminished Ca^{2+} overload in DT40 cells and rendered them more resistant to apoptotic stimuli [135]. Furthermore, expression of the C-terminal channel domain, an IP_3R1 cleavage product, increased the tendency of these cells to undergo apoptosis. IP_3R1 cleavage by caspase 3 was further shown to play an important role in apoptotic events that take place during mouse oocyte maturation [136,137]. However, other groups made observations contradicting the role of IP_3R1 as a relevant caspase 3 substrate. For instance, Guillemette and co-workers showed that HeLa or Jurkat cells exposed to a variety of apoptotic stimuli with robust caspase 3 activation did not display cleavage of IP_3R1 [138]. Here, recombinant caspase 3 also failed to cleave IP_3R1 in the microsomal fraction. Consistent with this, Boehning and co-workers showed that the staurosporine-induced rise in cytosolic [Ca^{2+}] in HeLa cells is

independent of caspase 3 expression or activity [139]. Also, IP_3R1 cleavage did not occur in staurosporine-treated MCF7 cells re-complemented with caspase 3. These contradicting results raise the question whether differing endogenous Bok levels may account for the varying sensitivity of IP_3R1 to caspase 3 cleavage in different cell models (e.g. DT40 cells versus HeLa or MCF7/caspase 3 cells).

Beclin 1

Beclin 1 is an important regulator of autophagy, a catabolic pathway in which damaged organelles and macromolecules are degraded and recycled [140]. This protein is localized within cytoplasmic structures, such as the ER, mitochondria and the perinuclear membrane. Beclin 1 is critical to initiate autophagy since it is part of one of the main signal-initiating complexes, consisting of class III phosphatidylinositol 3-kinase/Vps34, Beclin 1 and p150 protein [141]. The Beclin 1 protein is composed of three main domains: (i) a BH3 domain located at the N-terminus (a.a. 114-123) that interacts with anti-apoptotic Bcl-2-family members, (ii) a central coiled-coil domain (a.a. 144-269) mediating Beclin 1 self-association and dimerization, and (iii) a C-terminal evolutionarily conserved domain (a.a. 244-337) that enables protein interactions and binds lipid membranes of cell organelles [140–143]. Via these domains Beclin 1 interacts with co-factors, including Vps34, PKB/Akt, Bif-1, Bcl-2 and Bcl-XL, to positively regulate autophagy and initiate autophagosome formation [140]. Beclin 1 is a known tumor suppressor, since Beclin 1 haplodeficient mice display increased tumor formation [144]. Also, in ovarian, breast and prostate cancer, the tumor suppressive gene *BECN1* was mono-allelically deleted, whereas a decreased expression of Beclin 1 was observed in many types of cancer, including brain tumors and cervical cancer [140,141].

Several studies showed that IP_3Rs are directly targeted by Beclin 1, which has a prominent binding site in the suppressor domain of the IP_3R (a.a. 1-225) (Fig. 5) [145]. Originally, it was proposed that IP_3R served as a scaffold for Beclin 1, thereby inhibiting autophagy by making it less available for its autophagy-inducing role in the Vps34 complex [146]. This complex mediates one of the earlier steps within the autophagy process downstream of mTOR/unc-51-like kinase 1 complex but preceding the autophagosome formation. As such, the IP_3R served as a negative regulator of autophagy. In this context, Beclin 1 did not modulate IICR and thus IP_3Rs rather served as a sink for Beclin 1, thereby limiting Beclin 1 availability for driving the autophagy process. Further work actually found an autophagy-promoting role for the IP_3R/Beclin 1 complex [145]. Beclin 1 binding to the IP_3R occurred in a dynamic manner during starvation-induced autophagy, leading, together with an increased ER Ca^{2+}-store content, to a sensitization of agonist-induced Ca^{2+} signaling. Cells lacking Beclin 1 failed to display enhanced IP_3R-mediated Ca^{2+} signaling. This involved a direct sensitization of the IP_3R by Beclin 1, since purified Beclin 1 enhanced IICR in permeabilized cells. IP_3R sensitization and subsequent Ca^{2+} signaling were essential to drive starvation-induced autophagic flux, since pharmacological IP_3R inhibition and intracellular Ca^{2+} buffering prevented starvation-induced autophagy [145]. Yet, at this point it is not clear whether IP_3Rs and Ca^{2+} signaling are involved in Beclin 1's function as a tumor suppressor.

PKB/Akt and PTEN

The phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB)/Akt signaling pathway is a tightly controlled process regulating cell survival, proliferation and cell death [147,148]. PI3K, a lipid kinase, converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3) after it is activated by G-protein-coupled receptors and receptor tyrosine kinases [147,149]. Next, PIP_3 recruits phosphatidylinositol-dependent kinase 1 (PDK1) and PKB/Akt, a serine/threonine kinase, to the plasma membrane. This leads to PKB/Akt activation through phosphorylation of T308 by PDK1 and S473 by mTORC2. Subsequently, active PKB/Akt phosphorylates its substrates in the cytoplasm and nucleus, thereby regulating several cellular functions, including cell growth, proliferation, survival, metabolism, protein synthesis and apoptosis [147]. There are three Akt isoforms, Akt1, Akt2 and Akt3, which are structurally highly homologous but exhibit different functions [150]. Akt1 and Akt2 are ubiquitously expressed, whereas Akt3 expression is restricted to a few tissues. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a lipid and protein phosphatase, is the most important negative regulator of the PI3K/Akt signaling pathway [149]. Via its lipid phosphatase activity, PTEN reverses the action of PI3K by dephosphorylating PIP_3 to PIP_2, counteracting all downstream functions controlled by PKB/Akt. The protein phosphatase activity of PTEN is used to inhibit cell migration and cell cycle arrest [149]. The tumor suppressive activity of PTEN is lost in many human tumors, such as breast, thyroid, kidney, colorectal and prostate cancer [124,149]. Somatic mutations, gene silencing or epigenetic mechanisms cause this loss of functional PTEN in tumor cells. Hyperactivity of PKB/Akt, caused by mutations in the catalytic subunit or amplification of Akt, has also been observed in several cancer types [150]. For example, Akt1 amplification has been detected in gastric carcinomas, whereas amplification of Akt2 occurred in ovarian, breast, colorectal and pancreatic cancers. In several tumor types, including breast, colorectal, ovarian, lung and bladder cancer, an activating mutation on Akt1 (E17K), which results in constitutive localization of Akt at the plasma membrane and promotes growth factor-independent phosphorylation of T308 and S473, has been observed [150].

Over the past years, several studies reported the interaction of Akt kinase with IP_3Rs at the ER. Joseph and co-workers showed that phosphorylation of the IP_3R by PKB/Akt occurred *in vivo* [151]. All three IP_3R isoforms contain a consensus substrate motif for PKB/Akt kinase (R*X*R*XX*(S/T)) located in the cytosol-exposed C-terminal tail (Fig. 5). Upon Akt activation by insulin, endogenous IP_3Rs present in CHO-T cells were phosphorylated [151]. A prostate cancer cell line with constitutively active Akt due to loss of functional PTEN (LnCAP cells) exhibited constitutive phosphorylation of endogenous IP_3R1 as well. Exposure to a PI3K inhibitor removed IP_3R phosphorylation in the two aforementioned cell lines. In COS cells transfected with constitutively active Akt, IP_3R phosphorylation was inhibited by mutating a serine amino acid residue present in the Akt substrate motif of the IP_3R (S2681A and S2681E). IP_3R channel function was not directly modified by Akt phosphorylation, as IICR was comparable between wild-type, non-phosphorylatable S2681A mutant and phosphomimic S2681E mutant IP_3Rs expressed in COS cells [151]. Nevertheless, caspase 3 activation, induced by staurosporine, was enhanced in TKO-DT40 cells expressing the S2681A mutant compared with cells expressing wild-type IP_3Rs or IP_3R mutants with a mimicked effect of Akt phosphorylation (S2681E). In contrast, other studies did report reduced Ca^{2+} release from IP_3Rs phosphorylated by PKB/Akt [152,153]. It was shown that phosphorylation of IP_3Rs, induced by overexpressing constitutively active PKB/Akt, inhibited IICR and histamine-induced Ca^{2+} release, whereas the ER Ca^{2+} content was not decreased [152]. In this study, a greater ATP-induced Ca^{2+} response was observed in COS cells expressing mutant IP_3R^{S2681A} compared to cells expressing wild-

type IP_3R. The former were also more sensitive to apoptotic stimuli like menadione and displayed higher mitochondrial Ca^{2+} rises in response to these stimuli. Also, glioblastoma cells exhibiting Akt hyperactivity due to loss of PTEN showed reduced IICR, menadione-induced mitochondrial Ca^{2+} uptake and apoptosis compared with glioblastoma cells re-expressing PTEN [152]. These results indicate that phosphorylation of IP_3Rs by active PKB/Akt reduces Ca^{2+} release from the ER and subsequent Ca^{2+} transfer to the mitochondria, leading to the protection of cells from apoptotic stimuli (Fig. 3). Another independent study reported that agonist-induced Ca^{2+} release from the ER and the subsequent mitochondrial Ca^{2+} rise is reduced by expressing constitutively active Akt1 in HeLa cells, thereby protecting cells from apoptotic stimuli that act through mitochondrial Ca^{2+} overload like H_2O_2 [153]. This study elegantly showed that Akt modulated IP_3R activity, resulting in an inhibition of agonist-induced Ca^{2+} release and protection from Ca^{2+}-mediated apoptotic stimuli. In further work it was demonstrated that Akt reduces ER-mitochondrial Ca^{2+} transfer and protects against apoptosis by specifically acting on IP_3R3 [154]. Activated Akt suppressed IICR and apoptosis, induced by arachidonic acid, in COS7 cells, which lack detectable IP_3R1, but not in IP_3R3-deficient SH-SY5Y cells. However, Akt did reduce Ca^{2+} release and did protect against apoptotic stimuli in SH-SY5Y cells expressing IP_3R3. Interestingly, it has been proposed that pro-apoptotic ER-mitochondrial Ca^{2+} transfers preferentially occur via IP_3R3 [155,156], although a pivotal role of IP_3R1 in Ca^{2+}-mediated apoptotic cell death was reported in other studies as well [135,157].

Recently, tumor suppressor PTEN was found to be localized at the ER and MAMs and to modulate Ca^{2+} transfer from the ER to mitochondria in a protein phosphatase-dependent manner [158]. PTEN silencing, which leads to increased phosphorylation and activity of Akt, impaired the release of Ca^{2+} from the ER and lowered cytosolic and mitochondrial Ca^{2+} transients in response to agonists [158]. Consequently, sensitivity to arachidonic acid, an apoptotic inducer causing ER Ca^{2+} release, and subsequent mitochondrial Ca^{2+} overload were decreased in PTEN-silenced cells, but were both increased upon overexpression of PTEN that is targeted to the ER, indicating that ER-localized PTEN sensitizes cells to Ca^{2+}-mediated apoptotic stimuli (Fig. 3). Moreover, initiation of Ca^{2+}-dependent apoptosis with arachidonic acid enriched PTEN localization at the ER. Co-immunoprecipitation experiments revealed that PTEN interacts with IP_3R3 [158]. Finally, it was determined that ER-localized PTEN directly reduces the Akt-dependent phosphorylation state of the IP_3R through its protein phosphatase activity, hence modulating Ca^{2+} release from the ER and cellular sensitivity to Ca^{2+}-mediated apoptotic stimuli (Fig. 5).

Promyelocytic leukemia protein (PML)

The promyelocytic leukemia protein (PML) is an important tumor suppressor, which controls various cellular functions, including apoptosis, DNA-damage response, cellular proliferation and senescence [159,160]. The *PML* gene was identified through its location at the breakpoint of the t(15;17) chromosomal translocation, which is often observed in acute promyelocytic leukemia [160]. As a result of this translocation, the *PML* gene is juxtaposed to the gene encoding the retinoic acid receptor alpha, which leads to the formation of fusion proteins that hinder the differentiation of hematopoietic cells. At the structural level, the N-terminal part of the PML protein is highly structured, in contrast to its C-terminus, and contains a RING domain, which is a zinc-finger that exhibits E3 ligase activity, two additional zinc-finger motifs called B-boxes and an alpha-helical coiled-

coil domain [160,161]. These N-terminal domains are responsible for the oligomerization of PML and they mediate protein-protein interactions, allowing PML to concentrate in nuclear bodies. To exert its tumor suppressive function, PML interacts with a large number of proteins, including sumoylated PML, p53, protein phosphatase 2A (PP2A) and PKB/Akt [160,161]. PML expression is lost or reduced in hematopoietic malignancies as well as in solid tumors, such as colon, lung, prostate and breast cancers [160]. Interestingly, PML-knockout mice and primary cells lacking PML are protected from apoptosis triggered by a variety of stimuli [162].

A few years ago, extra-nuclear PML was discovered to be localized to the ER and MAMs and to regulate apoptosis at the ER by modulating Ca^{2+} release [163]. Cytosolic and mitochondrial Ca^{2+} responses induced by agonists or oxidative apoptotic stimuli were higher in cells expressing PML than in cells lacking PML. Consistently, the presence of PML in cells was protective against ER stress-induced apoptosis [163]. Interestingly, the dampened Ca^{2+} responses observed in PML-deficient cells could be fully restored by ER-targeted PML [163]. As a consequence, these cells regained sensitivity to ER stress-induced apoptosis, but remained resistant to etoposide, an apoptotic inducer that acts independently of Ca^{2+}. These functional observations could be linked to a physical interaction between PML and IP_3R3 [163]. Moreover, the levels of phosphorylated IP_3R3 were higher in $PML^{-/-}$ than in $PML^{+/+}$ MEFs cells due to higher amounts of phosphorylated, active Akt and reduced amounts of protein phosphatase PP2A associated with IP_3R3 in the former [163]. Hence, the tumor suppressor PML was found to be essential to recruit PP2A to IP_3R3-Akt complexes at the MAMs (Fig. 3), where PP2A counteracts Akt activity, thereby suppressing Akt-mediated phosphorylation of the IP_3R3 (Fig. 5). As a consequence, IP_3R3-mediated Ca^{2+} transfer from the ER into the mitochondria and, eventually, apoptosis are promoted in PML-expressing cells [161,163]. Thus, similarly to PTEN, PML stimulates pro-apoptotic Ca^{2+} signaling events by negatively regulating the activity of Akt at the ER.

Breast and ovarian cancer susceptibility gene 1 (BRCA1)

Tumor suppressor BRCA1 is a chromatin-interacting protein that has a critical role in homologous recombination repair of double-strand DNA breaks [164]. Its main function is to preserve genomic integrity, although growing evidence indicates that BRCA1 activity is necessary for mammary epithelial differentiation as well [164]. Breast and ovarian cancer are frequently associated with impaired functioning of BRCA1, caused by BRCA1 mutations, loss of expression or BRCA1 downregulation. BRCA1 mutations predispose to breast and ovarian cancer, although it is still not clear why this predisposition exists since this tumor suppressor is expressed in all cells. In families with hereditary breast and ovarian cancer, the N-terminal RING domain and the C-terminal BRCT repeats of BRCA1 are frequently mutated [164]. These two domains contribute to the tumor suppressive activity of BRCA1. The N-terminal RING domain exhibits E3 ubiquitin ligase activity when heterodimerized with BARD1, another RING- and BRCT domain-containing protein [164,165]. The BRCT repeats are needed to concentrate BRCA1 in subnuclear repair foci that appear after DNA damage. These repeats are phosphopeptide-binding domains responsible for the interaction of BRCA1 with partner proteins like the endonuclease CtIP [166], the adaptor protein Abraxas [167] and the DNA helicase BRIP1 [168]. Through their interaction with BRCA1, these partner proteins regulate DNA repair and maintain genome stability.

Recently, BRCA1 was identified as a novel binding partner of the IP_3R1 [169]. A major subpopulation of the BRCA1 pool localized outside the nucleus, residing at ER membranes by binding phospholipids. Full-length IP_3R/BRCA1 complexes were identified. The complex was formed through a direct interaction of the C-terminal tail of the IP_3R1 with the N-terminal domain of the BRCA1 protein (a.a. 1-112, the so-called RING domain) (Fig. 5). BRCA1 overexpression caused an increase in IP_3R-mediated Ca^{2+} signaling in intact HeLa cells, particularly sensitizing cells towards low [agonist] [169]. This was due to a direct sensitization of IP_3R channels by BRCA1 via its RING domain, which was sufficient by itself to increase IP_3R single-channel activity (Fig. 3). FRET studies revealed that the IP_3R1/BRCA1-protein complex was dynamically formed during paclitaxel-induced apoptosis [169]. Furthermore, in an ovarian cancer cell line paclitaxel-induced cytosolic Ca^{2+} rise and apoptotic susceptibility were critically dependent on the BRCA1-expression status. Comparison of wild-type *versus* TKO-DT40 cells revealed that the recruitment of BRCA1 to the ER membranes increased in an IP_3R-dependent manner during apoptosis [169]. However, also other mechanisms appeared to contribute to the recruitment of BRCA1 to ER membranes, likely involving the C-terminal domain of BRCA1 (the BRCT repeats), which displayed an intrinsic lipid-binding property [169]. The BRCT repeats, but not the N-terminal RING domain, bound several ER-resident phospholipids, including phosphatidic acid and PIP_2. Further studies will be needed to determine whether BRCA1's pro-apoptotic function is dependent on the presence of the IP_3R, e.g. by comparing the effect of BRCA1 overexpression in wild-type versus TKO-DT40 cells.

p53

The transcription factor p53 is one of the most important tumor suppressor proteins in the cell. The p53 protein transcriptionally regulates its downstream target genes, preventing tumorigenesis [170]. Multiple biological processes are regulated by p53, including cell cycle arrest, apoptosis, senescence, DNA repair and energy metabolism [171]. In unstressed cells, the level of p53 is kept low through proteasomal degradation of the protein. In response to stress signals, such as DNA damage, hypoxia and oncogene activation, p53 is stabilized principally through post-translational modifications, leading to p53 activation and accumulation in the cell. Consequently, activated p53 regulates the expression of its target genes by binding to a specific degenerate DNA sequence, known as the p53-responsive element. In approximately 50% of all human tumors and in almost every type of cancer, including leukemia, lung, colorectal, cervical and ovarian cancer, p53 mutations, which are mainly missense mutations, occur [170–172]. These mutant p53 proteins either lose the tumor suppressive functions of wild-type p53 or acquire new oncogenic activities that are independent of wild-type p53, such as promoting angiogenesis, metastasis and tumor cell proliferation [170].

A recent study revealed a novel, non-transcriptional role for cytosolic p53 at the level of the ER [173,174]. Under untreated conditions p53 was found to localize at the ER, the MAMs and the cytosol [173]. After p53 induction in response to stress, elicited by chemotherapeutics like adriamycin or oxidative stress like H_2O_2, p53 accumulated at the ER and the MAMs. Furthermore, apoptotic cell death, triggered by H_20_2, was enhanced after adriamycin-induced p53 accumulation at the ER and the MAMs [173]. This correlated to an altered regulation of Ca^{2+} homeostasis after p53 induction at the ER/MAM compartments. The elevated ER Ca^{2+} levels and agonist-induced Ca^{2+} release by adriamycin was critically dependent on the cellular presence of p53 and promoted

apoptosis. Using pharmacological compounds inhibiting p53's transcriptional function and p53-targeted chimeras (p53-ΔNLS and ER-p53), it was excluded that the p53-dependent modulation on Ca^{2+} homeostasis was caused by a transcriptional role of p53 [173]. Instead, p53 modulated Ca^{2+} homeostasis and apoptosis through an interaction of its C-terminal regulatory domain with the SERCA pump at the ER. In cells overexpressing wild-type p53, the rate of Ca^{2+} accumulation in the ER increased proportionally with the induction of p53 by adriamycin, indicating that p53 promoted SERCA activity. Interestingly, oncogenic p53 mutants failed to stimulate SERCA activity. The underlying molecular mechanism involved the lowering of the oxidation state of SERCA upon p53 activation, leading to increased Ca^{2+} loading of the ER (Fig. 2) [173]. As a consequence, Ca^{2+} transfer from the ER to the mitochondria is boosted, resulting in a higher level of mitochondrial Ca^{2+} overload and apoptotic cell death. The interplay between p53 and Ca^{2+} signaling is not limited to chemotherapy but is also an inherent part of the cellular response of cancer cells exposed to photodynamic therapy (PDT) [175]. In MEF cells, PDT transiently increased the cytosolic and mitochondrial [Ca^{2+}] in a p53-dependent manner. Consequently, $p53^{-/-}$ MEFs were more resistant to apoptosis triggered by PDT compared with the $p53^{+/+}$ clone. This indicates that p53 is required to regulate the efficacy of apoptotic Ca^{2+} signals *in vitro*, suggesting that cancer cells can become resistant to PDT by mutation or loss of p53. These concepts were also validated by *in vivo* Ca^{2+} signaling and cell death measurements in three-dimensional tumor masses [175]. PDT triggered higher cytosolic Ca^{2+} responses in $p53^{+/+}$ than in $p53^{-/-}$ xenografted tumors, indicating that functional p53 is needed to generate an efficient Ca^{2+} response after PDT to trigger apoptotic cell death in tumor cells. Moreover, the sensitivity to PDT-triggered apoptosis was reduced in tumor masses expressing p53 by buffering intracellular Ca^{2+} [175]. Oppositely, the PDT-induced Ca^{2+} response and apoptosis in p53-deficient cells could be restored by overexpressing either MCU or SERCA. Hence, both *in vitro* and *in vivo,* extra-nuclear p53 promotes pro-apoptotic Ca^{2+} signaling that originates at the ER, thereby determining the sensitivity of tumor cells to PDT.

Furthermore, these studies also provide novel insights in the mechanisms that underlie the poor responses of cancer cells deficient in p53 to chemotherapy or PDT [176,177], linking the absence of p53 to a failure to cause pro-apoptotic Ca^{2+} transmission from ER to mitochondria and to kill cancer cells. Hence, boosting the efficiency of ER-mitochondrial Ca^{2+} transfer may therefore offer a potential strategy to enhance the responses of cancer cells to chemo- and photo-therapeutic treatments [176,177].

Ras

Ras is a small GTPase that regulates various intracellular signaling pathways, thereby controlling cell growth, differentiation and survival [178,179]. There are three Ras isoforms, K-Ras, N-Ras and H-Ras, which are extremely homologous to one another. The Ras protein, anchored to the inner leaflet of the plasma membrane, is activated by cell surface receptors, including G-protein-coupled receptors and receptor tyrosine kinases, which induce guanine nucleotide exchange factors to interchange GDP with GTP on Ras [179,180]. Ras activity is kept under control by GTPase-activating proteins (GAPs), which inactivate Ras by enabling its GTPase activity, resulting in the hydrolysis of GTP. Active Ras controls cell fate via many downstream effectors, such as Raf, PLCε and PI3Ks [179]. The oncogene Ras is mutated in 33% of human cancers, including pancreatic, colorectal and lung cancer [180]. Due

to mutational activation, Ras becomes constitutively active by binding GTP in an unregulated manner instead of switching between the active and inactive states like wild-type Ras. Especially mutations of a.a. 12, 13 or 61 render Ras insensitive to GAP activity and reduce the intrinsic GTPase activity.

Oncogenic Ras has been implicated in regulating IP_3Rs and Ca^{2+} signaling. Earlier work indicated that Ras could enhance agonist-induced Ca^{2+} signaling in fibroblasts and other cell types upon Ras transformation [181]. This may be linked to the ability of Ras to directly interact with PLCε when GTP analogues were present [182]. PLCε appeared to be recruited to the plasma membrane in a Ras-dependent manner, resulting in an increased enzyme activity responsible for PIP_2 hydrolysis and the production of IP_3. Ras could *in vitro* not induce an increase in PLCε activity, indicating that Ras may stimulate PLCε by causing its translocation to the plasma membrane. Further molecular studies revealed a prominent role for the Ras-associating domain 2 (RA2), but not RA1, within the PLCε structure for the regulation by Ras [183]. Interaction of Ras with the RA2 domain of PLCε enabled its translocation to the plasma membrane and altered its conformation from an auto-inhibited state into an active state in which PIP_2 is available to the catalytic site, resulting in PIP_2 hydrolysis and IP_3 production.

Besides targeting IP_3-producing enzymes, Ras has also been implicated in altering IP_3R-expression profiles. This was shown in a study comparing two isogenic colorectal cancer cell lines: one cell line expressing oncogenic mutant K-Ras^{G13D} and one cell line expressing wild-type K-Ras [184]. Cells expressing K-Ras^{G13D} displayed a decrease in agonist-induced Ca^{2+} signaling, while silencing of Ras in these cells enhanced agonist-induced Ca^{2+} signaling [184]. This effect appeared to be a direct effect on the IP_3R, since a membrane-permeable variant of IP_3 had similar effects as agonists that indirectly lead to an increase in the intracellular IP_3 levels. The remodeling of IP_3-induced Ca^{2+} signaling by K-Ras^{G13D} occurred at the level of the ER, since K-Ras^{G13D}-expressing cells displayed a decrease in ER Ca^{2+}-store content [184]. This correlated with alterations in the expression levels of key ER Ca^{2+}-transport systems, in which K-Ras^{G13D}-expressing cells expressed lower SERCA2b and IP_3R3 levels but considerably higher IP_3R1 levels. Thus, a decreased ER Ca^{2+} uptake by SERCA2b downregulation and an increase in ER Ca^{2+} leak by IP_3R1 upregulation may account for the decrease in steady state ER Ca^{2+} levels in these cells (Fig. 2). Furthermore, IP_3R3 has been proposed as a pro-apoptotic factor by specifically mediating the transfer of pro-apoptotic Ca^{2+} signals from the ER into the mitochondria, resulting in mitochondrial Ca^{2+} overload [156]. In any case, both the decreased ER Ca^{2+} levels and the decreased IP_3R3-expression levels, would be protective mechanisms preventing mitochondrial Ca^{2+} overload and favoring apoptosis resistance. This is backed up by experiments showing that K-Ras^{G13D} cells displayed reduced mitochondrial Ca^{2+} signals in response to agonists and an increased resistance towards apoptotic stimuli [184]. In summary, this study convincingly shows that Ras not only impacts Ca^{2+} signaling at the level of IP_3-producing enzymes but also directly at the level of the ER by remodeling the expression of IP_3R isoforms and SERCA2b. As such, oncogenic mutations in K-Ras may favor survival of cancer cells by dampening ER-originating Ca^{2+} signaling events that stimulate the apoptotic sensitivity of cells, promoting malignant cell survival.

In addition to this, it is known that protein kinase C (PKC)-mediated phosphorylation of K-Ras4B causes its translocation from the plasma membrane to the intracellular environment, including the ER membranes, and limits cell survival [185]. A recent study revealed that Ras can impact cell survival by targeting the IP_3R at the ER [186]. Strikingly, cell lines expressing wild-type K-Ras were sensitive to PKC agonists, while cell lines expressing the K-Ras^{G13D} mutant were much more resistant to this

treatment. A phosphomimetic mutant of activated K-Ras could bind the C-terminus of the IP_3R1 with much greater efficiency than activated K-Ras or the phosphorylation dead mutant of activated K-Ras [186]. Furthermore, recombinant Bcl-XL enhanced the binding of the phosphomimetic K-Ras variant to the C-terminus of the IP_3R1. Also endogenously activated K-Ras interacted with IP_3R3, a phenomenon enhanced by PKC agonists. Interestingly, at the IP_3R3 single-channel level, purified activated phosphomimetic K-Ras, but not the activated phosphodead K-Ras, counteracted the sensitization of IP_3Rs by purified Bcl-XL (Fig. 5) [186]. In intact cells, overexpression of activated K-Ras suppressed mitochondrial Ca^{2+} dynamics, a phenomenon aggravated when overexpressing activated phosphomimetic K-Ras (Fig. 4). The impact of this K-Ras overexpression on mitochondrial Ca^{2+} was strictly dependent on Bcl-XL, since overexpression in Bcl-XL-knockout cells was without effect [186]. This impacted cell survival and death processes, like autophagy. Hence, overexpression of activated phosphomimetic K-Ras or exposing cancer cells that express endogenously activated K-Ras to PKC activators resulted in cell death and increased autophagosome punctae formation. This depended on the presence of Bcl-XL, since Bcl-XL-deficient cells were resistant to activated phosphomimetic K-Ras overexpression [186]. This autophagy stimulation appeared to promote cell death, since cells lacking the essential autophagy gene *ATG5* were partially protected against phosphomimetic K-Ras overexpression, supporting the concept that excessive autophagy activation can result in cell death. Interestingly, a recent study illustrated a direct link between Ca^{2+} regulation and another oncogenic form of Ras: the oncogenic H-Ras (H-Ras12v). H-Ras localizes at the MAMs and regulates the transmission of Ca^{2+} from the ER to mitochondria, thereby inhibiting apoptosis and in turn promoting cellular transformation [187].

To summarize, K-Ras and its oncogenic forms can alter cell survival by modulating the properties of the ER Ca^{2+} stores, lowering the ER Ca^{2+}-store content and IP_3R3 levels, two protective mechanisms implicated in apoptosis resistance. In addition, phosphorylation of K-Ras results in cell death by suppressing pro-survival Ca^{2+}-signaling events in the mitochondria and triggering excessive autophagy as a cell-death mechanism.

Conclusions

A growing list of tumor suppressors and oncogenes that have additional roles in the regulation of ER Ca^{2+} handling have been identified. These functions appear to be critical for their biological activity of controlling cell death and survival. At the level of the ER, tumor suppressors and oncogenes impact Ca^{2+} homeostasis (e.g. ER Ca^{2+}-store content) and/or Ca^{2+} dynamics (e.g. Ca^{2+} release from the ER), thereby facilitating or preventing mitochondrial Ca^{2+} overload, respectively. In many cancers, in which these tumor suppressors and oncogenes are dysregulated, Ca^{2+} homeostasis and dynamics are altered in such a way that cells fail to induce pro-apoptotic Ca^{2+} signaling events that lead to mitochondrial Ca^{2+} overload. As a consequence, in these cancer cells apoptosis is, at least partly, avoided by an altered Ca^{2+} signaling, which favors persistent survival despite on-going cell stress and damage. Restoring proper Ca^{2+} signaling could therefore be a promising avenue for re-sensitizing cancer cells to their intrinsic pro-apoptotic signaling mechanisms and to therapeutic interventions like chemotherapy and PDT.

Acknowledgements

J.B.P. and G.B. are supported by grants from the Fund for Scientific Research - Flanders (FWO) (grants G.0571.12N, G.0634.13N, G.0C91.14N, G.0927.15N and G.0A34.16N) and the Research Council – KU Leuven (OT14/101). P.P. is grateful to Camilla degli Scrovegni for continuous support. P.P. lab is supported by the Italian Association for Cancer Research (AIRC: IG-14442), local funds from the University of Ferrara, Telethon (GGP15219/B), the Italian Ministry of Health, the Italian Ministry of Education and University and Research (COFIN n. 20129JLHSY_002, FIRB n. RBAP11FXBC_002, and Futuro in Ricerca n. RBFR10EGVP_001). M.B. is holder of a Ph.D. Fellowship from the FWO.

References

[1] M. J. Berridge, P. Lipp, and M. D. Bootman, "The versatility and universality of calcium signalling," *Nat. Rev. Mol. Cell Biol.*, vol. 1, no. 1, pp. 11–21, 2000.

[2] M. J. Berridge, M. D. Bootman, and H. L. Roderick, "Calcium signalling: dynamics, homeostasis and remodelling," *Nat. Rev. Mol. Cell Biol.*, vol. 4, no. 7, pp. 517–529, 2003.

[3] T. Pozzan, R. Rizzuto, P. Volpe, and J. Meldolesi, "Molecular and cellular physiology of intracellular calcium stores," *Physiol Rev*, vol. 74, no. 3, pp. 595–636, 1994.

[4] D. E. Clapham, "Calcium signaling," *Cell*, vol. 131, no. 6, pp. 1047–1058, 2007.

[5] G. Csordás, P. Várnai, T. Golenár, S. Roy, G. Purkins, T. G. Schneider, T. Balla, and G. Hajnóczky, "Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface," *Mol. Cell*, vol. 39, no. 1, pp. 121–132, 2010.

[6] M. Giacomello, I. Drago, M. Bortolozzi, M. Scorzeto, A. Gianelle, P. Pizzo, and T. Pozzan, "Ca^{2+} hot spots on the mitochondrial surface are generated by Ca^{2+} mobilization from stores, but not by activation of store-operated Ca^{2+} channels," *Mol. Cell*, vol. 38, no. 2, pp. 280–290, 2010.

[7] D. Prins and M. Michalak, "Organellar calcium buffers," *Cold Spring Harb. Perspect. Biol.*, vol. 3, no. 3, pp. 1–16, 2011.

[8] I. Vandecaetsbeek, P. Vangheluwe, L. Raeymaekers, F. Wuytack, and J. Vanoevelen, "The Ca^{2+} pumps of the endoplasmic reticulum and Golgi apparatus," *Cold Spring Harb. Perspect Biol*, vol. 3, no. 5: a004184, 2011.

[9] J. K. Foskett, C. White, K. Cheung, and D. D. Mak, "Inositol trisphosphate receptor Ca^{2+} release channels," *Physiol. Rev.*, vol. 87, no. 2, pp. 593–658, 2007.

[10] J. B. Parys and H. De Smedt, "Inositol 1,4,5-trisphosphate and its receptors," *Adv. Exp. Med. Biol.*, vol. 740, pp. 255–279, 2012.

[11] O. A. Fedorenko, E. Popugaeva, M. Enomoto, P. B. Stathopulos, M. Ikura, and I. Bezprozvanny, "Intracellular calcium channels: Inositol-1,4,5-trisphosphate receptors," *Eur. J. Pharmacol.*, vol. 739, pp. 39–48, 2014.

[12] I. Bezprozvanny, J. Watras, and B. E. Ehrlich, "Bell-shaped calcium-response curves of $Ins(1,4,5)P_3$- and calcium-gated channels from endoplasmic reticulum of cerebellum," *Nature*, vol. 351, pp. 751–754, 1991.

[13] E. Finch, T. Turner, and S. Goldin, "Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release," *Science*, vol. 252, pp. 443–446, 1991.

[14] M. Iino, "Biphasic Ca^{2+} dependence of inositol 1,4,5-trisphosphate-induced Ca^{2+} release in smooth muscle cells of the guinea pig taenia caeci," *J. Gen. Physiol.*, vol. 95, no. 6, pp. 1103–1122, 1990.

[15] C. U. Choe and B. E. Ehrlich, "The inositol 1,4,5-trisphosphate receptor (IP_3R) and its regulators: sometimes good and sometimes bad teamwork," *Sci. STKE*, pp. 1–12, 2006.

[16] V. Vanderheyden, B. Devogelaere, L. Missiaen, H. De Smedt, G. Bultynck, and J. B. Parys, "Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release by reversible phosphorylation and dephosphorylation," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1793, no. 6, pp. 959–970, 2009.

[17] M. Iino, "Effects of adenine nucleotides on inositol 1,4,5-trisphosphate-induced calcium release in vascular smooth muscle cells," *J Gen Physiol*, vol. 98, no. 4, pp. 681–698, 1991.

[18] I. Bezprozvanny and B. E. Ehrlich, "ATP modulates the function of inositol 1,4,5-trisphosphate-gated channels at two sites.," *Neuron*, vol. 10, no. 6, pp. 1175–1184, 1993.

[19] K. Maes, L. Missiaen, P. De Smet, S. Vanlingen, G. Callewaert, J. B. Parys, and H. De Smedt, "Differential modulation of inositol 1,4,5-trisphosphate receptor type 1 and type 3 by ATP," *Cell Calcium*, vol. 27, no. 5, pp. 257–267, 2000.

[20] M. J. Betzenhauser, L. E. Wagner, M. Iwai, T. Michikawa, K. Mikoshiba, and D. I. Yule, "ATP modulation of Ca^{2+} release by type-2 and type-3 inositol (1,4,5)-triphosphate receptors:

differing ATP sensitivities and molecular determinants of action," *J. Biol. Chem.*, vol. 283, no. 31, pp. 21579–21587, 2008.

[21] S. Marchi, S. Patergnani, and P. Pinton, "The endoplasmic reticulum-mitochondria connection: One touch, multiple functions," *Biochim. Biophys. Acta - Bioenerg.*, vol. 1837, no. 4, pp. 461–469, 2014.

[22] R. Rizzuto, D. De Stefani, A. Raffaello, and C. Mammucari, "Mitochondria as sensors and regulators of calcium signalling," *Nat. Rev. Mol. Cell Biol.*, vol. 13, no. 9, pp. 566–578, 2012.

[23] R. Rizzuto, S. Marchi, M. Bonora, P. Aguiari, A. Bononi, D. De Stefani, C. Giorgi, S. Leo, A. Rimessi, R. Siviero, E. Zecchini, and P. Pinton, "Ca^{2+} transfer from the ER to mitochondria: When, how and why," *Biochim. Biophys. Acta - Bioenerg.*, vol. 1787, no. 11, pp. 1342–1351, 2009.

[24] V. Shoshan-Barmatz and D. Gincel, "The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death," *Cell Biochem. Biophys.*, vol. 39, no. 3, pp. 279–292, 2003.

[25] M. Colombini, "VDAC structure, selectivity, and dynamics," *Biochim. Biophys. Acta - Biomembr.*, vol. 1818, no. 6, pp. 1457–1465, 2012.

[26] S. Marchi and P. Pinton, "The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications," *J. Physiol.*, vol. 592, no. 5, pp. 829–839, 2014.

[27] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M. R. Wieckowski, D. Cavagna, A. Nagy, T. Balla, and R. Rizzuto, "Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels," *J. Cell Biol.*, vol. 175, no. 6, pp. 901–911, 2006.

[28] A. R. van Vliet, T. Verfaillie, and P. Agostinis, "New functions of mitochondria associated membranes in cellular signaling," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1843, no. 10, pp. 2253–2262, 2014.

[29] C. Cárdenas, R. A. Miller, I. Smith, T. Bui, J. Molgó, M. Müller, H. Vais, K. H. Cheung, J. Yang, I. Parker, C. B. Thompson, M. J. Birnbaum, K. R. Hallows, and J. K. Foskett, "Essential regulation of cell bioenergetics by constitutive $InsP_3$ receptor Ca^{2+} transfer to mitochondria," *Cell*, vol. 142, no. 2, pp. 270–283, 2010.

[30] C. Wiel, H. Lallet-Daher, D. Gitenay, B. Gras, B. Le Calvé, A. Augert, M. Ferrand, N. Prevarskaya, H. Simonnet, D. Vindrieux, and D. Bernard, "Endoplasmic reticulum calcium release through ITPR2 channels leads to mitochondrial calcium accumulation and senescence," *Nat. Commun.*, vol. 5, pp. 3792–3793, 2014.

[31] A. Rimessi, M. Bonora, S. Marchi, S. Patergnani, C. M. T. Marobbio, F. M. Lasorsa, and P. Pinton, "Perturbed mitochondrial Ca^{2+} signals as causes or consequences of mitophagy induction," *Autophagy*, vol. 9, no. 11, pp. 1677–1686, 2013.

[32] C. Cárdenas and J. K. Foskett, "Mitochondrial Ca^{2+} signals in autophagy," *Cell Calcium*, vol. 52, no. 1, pp. 44–51, 2012.

[33] J. P. Decuypere, G. Monaco, G. Bultynck, L. Missiaen, H. De Smedt, and J. B. Parys, "The IP_3 receptor-mitochondria connection in apoptosis and autophagy," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1813, no. 5, pp. 1003–1013, 2011.

[34] R. J. Kaufman and J. D. Malhotra, "Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1843, no. 10, pp. 2233–2239, 2014.

[35] C. Giorgi, F. Baldassari, A. Bononi, M. Bonora, E. De Marchi, S. Marchi, S. Missiroli, S. Patergnani, A. Rimessi, J. M. Suski, M. R. Wieckowski, and P. Pinton, "Mitochondrial Ca^{2+} and apoptosis," *Cell Calcium*, vol. 52, no. 1, pp. 36–43, 2012.

[36] G. Morciano, C. Giorgi, M. Bonora, S. Punzetti, R. Pavasini, M. R. Wieckowski, G. Campo, and P. Pinton, "Molecular identity of the mitochondrial permeability transition pore and its role in ischemia-reperfusion injury," *J. Mol. Cell. Cardiol.*, vol. 78, pp. 142–153, 2015.

[37] M. S. Hwang, C. T. Schwall, E. Pazarentzos, C. Datler, N. N. Alder, and S. Grimm, "Mitochondrial Ca^{2+} influx targets cardiolipin to disintegrate respiratory chain complex II for cell death induction," *Cell Death Differ.*, vol. 21, no. 11, pp. 1–13, 2014.

[38] J. B. Parys, J. P. Decuypere, and G. Bultynck, "Role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+}-release channel in autophagy," *Cell Commun. Signal.*, vol. 10, no. 1, p. 17, 2012.

[39] J. P. Decuypere, J. B. Parys, and G. Bultynck, "ITPRs/inositol 1,4,5-trisphosphate receptors in autophagy: From enemy to ally," *Autophagy*, vol. 11, no. 10, pp. 1944-1948, 2015.

[40] E. Kania, B. Pająk, and A. Orzechowski, "Calcium homeostasis and ER stress in control of autophagy in cancer cells," *Biomed Res. Int.*, vol. 2015, pp. 1–12, 2015.

[41] V. Sica, L. Galluzzi, J. M. Bravo-San Pedro, V. Izzo, M. C. Maiuri, and G. Kroemer, "Organelle-specific initiation of autophagy," *Mol. Cell*, vol. 59, no. 4, pp. 522–539, 2015.

[42] J. P. Decuypere, G. Bultynck, and J. B. Parys, "A dual role for Ca^{2+} in autophagy regulation," *Cell Calcium*, vol. 50, no. 3, pp. 242–250, 2011.

[43] M. Høyer-Hansen, L. Bastholm, P. Szyniarowski, M. Campanella, G. Szabadkai, T. Farkas, K. Bianchi, N. Fehrenbacher, F. Elling, R. Rizzuto, I. S. Mathiasen, and M. Jäättelä, "Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2," *Mol. Cell*, vol. 25, no. 2, pp. 193–205, 2007.

[44] T. A. Stewart, K. T. D. S. Yapa, and G. R. Monteith, "Altered calcium signaling in cancer cells," *Biochim. Biophys. Acta - Biomembr.*, vol. 1848, no. 10, pp. 2502–2511, 2014.

[45] N. Prevarskaya, R. Skryma, and Y. Shuba, "Calcium in tumour metastasis: new roles for known actors," *Nat. Rev. Cancer*, vol. 11, no. 8, pp. 609–618, 2011.

[46] H. L. Roderick and S. J. Cook, "Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival," *Nat. Rev. Cancer*, vol. 8, no. 5, pp. 361–375, 2008.

[47] G. R. Monteith, F. M. Davis, and S. J. Roberts-Thomson, "Calcium channels and pumps in cancer: Changes and consequences," *J. Biol. Chem.*, vol. 287, no. 38, pp. 31666–31673, 2012.

[48] S. Marchi, C. Giorgi, M. Oparka, J. Duszynski, M. R. Wieckowski, and P. Pinton, "Oncogenic and oncosuppressive signal transduction at mitochondria-associated endoplasmic reticulum membranes," *Mol. Cell. Oncol.*, vol. 1, no. 2: e956469, 2014.

[49] H. Akl, T. Vervloessem, S. Kiviluoto, M. Bittremieux, J. B. Parys, H. De Smedt, and G. Bultynck, "A dual role for the anti-apoptotic Bcl-2 protein in cancer: Mitochondria versus endoplasmic reticulum," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1843, no. 10, pp. 2240–2252, 2014.

[50] E. F. Greenberg, A. R. Lavik, and C. W. Distelhorst, "Bcl-2 regulation of the inositol 1,4,5-trisphosphate receptor and calcium signaling in normal and malignant lymphocytes: Potential new target for cancer treatment," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1843, no. 10, pp. 2205–2210, 2014.

[51] J. K. Brunelle and A. Letai, "Control of mitochondrial apoptosis by the Bcl-2 family.," *J. Cell Sci.*, vol. 122, no. 4, pp. 437–441, 2009.

[52] J. E. Chipuk, T. Moldoveanu, F. Llambi, M. J. Parsons, and D. R. Green, "The BCL-2 family reunion," *Mol. Cell*, vol. 37, no. 3, pp. 299–310, 2010.

[53] E. Szegezdi, D. C. Macdonald, T. Ní Chonghaile, S. Gupta, and A. Samali, "Bcl-2 family on guard at the ER," *Am. J. Physiol. Cell Physiol.*, vol. 296, no. 5, pp. C941–C953, 2009.

[54] L. Pegoraro, A. Palumbo, J. Erikson, M. Falda, B. Giovanazzo, B. S. Emanuel, G. Rovera, P. C. Nowell, and C. M. Croce, "A 14;18 and an 8;14 chromosome translocation in a cell line derived from an acute B-cell leukemia," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 81, no. 22, pp. 7166–7170, 1984.

[55] Y. Tsujimoto, L. R. Finger, J. Yunis, P. C. Nowell, and C. M. Croce, "Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation," *Science*, vol. 226, no. 4678, pp. 1097–1099, 1984.

[56] Q. Chen, H. Xu, A. Xu, T. Ross, E. Bowler, Y. Hu, and E. J. Lesnefsky, "Inhibition of Bcl-2 sensitizes mitochondrial permeability transition pore (MPTP) opening in ischemia-damaged mitochondria," *PLoS One*, vol. 10, no. 3: e0118834, 2015.

[57] T. Moldoveanu, A. V. Follis, R. W. Kriwacki, and D. R. Green, “Many players in BCL-2 family affairs,” *Trends Biochem. Sci.*, vol. 39, no. 3, pp. 101–111, 2014.

[58] M. Adachi, J. Cossman, D. Longo, C. M. Croce, and Y. Tsujimoto, “Variant translocation of the bcl-2 gene to immunoglobulin lambda light chain gene in chronic lymphocytic leukemia,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 86, no. 8, pp. 2771–2774, 1989.

[59] A. Aisenberg, B. Wilkes, and J. Jacobson, “The bcl-2 gene is rearranged in many diffuse B-cell lymphomas,” *Blood*, vol. 71, no. 4, pp. 969–972, 1988.

[60] J. C. Daniel and W. R. Smythe, “The role of bcl-2 family members in non-small cell lung cancer,” *Semin. Thorac. Cardiovasc. Surg.*, vol. 16, no. 1, pp. 19–27, 2004.

[61] Y. Tsujimoto, J. Cossman, E. Jaffe, and C. M. Croce, “Involvement of the Bcl-2 gene in human follicular lymphoma,” *Science*, vol. 228, no. 4706, pp. 1440–1443, 1985.

[62] G. A. Calin, Y. Pekarsky, and C. M. Croce, “The role of microRNA and other non-coding RNA in the pathogenesis of chronic lymphocytic leukemia,” *Best Pract. Res. Clin. Haematol.*, vol. 20, no. 3, pp. 425–437, 2007.

[63] M. Hanada, D. Delia, A. Aiello, E. Stadtmauer, and J. C. Reed, “Bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia,” *Blood*, vol. 82, no. 6, pp. 1820–1828, 1993.

[64] P. Pinton, D. Ferrari, P. Magalhães, K. Schulze-osthoff, F. Di Virgilio, T. Pozzan, and R. Rizzuto, “Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells,” *Cell*, vol. 148, no. 5, pp. 857–862, 2000.

[65] R. Foyouzi-Youssefi, S. Arnaudeau, C. Borner, W. L. Kelley, J. Tschopp, D. P. Lew, N. Demaurex, and K. H. Krause, “Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 11, pp. 5723–5728, 2000.

[66] A. E. Palmer, C. Jin, J. C. Reed, and R. Y. Tsien, “Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, no. 50, pp. 17404–17409, 2004.

[67] S. L. Schendel, Z. Xie, M. O. Montal, S. Matsuyama, M. Montal, and J. C. Reed, “Channel formation by antiapoptotic protein Bcl-2,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, no. 10, pp. 5113–5118, 1997.

[68] S. A. Oakes, L. Scorrano, J. T. Opferman, M. C. Bassik, M. Nishino, T. Pozzan, and S. J. Korsmeyer, “Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 102, no. 1, pp. 105–110, 2005.

[69] E. F. Eckenrode, J. Yang, G. V. Velmurugan, J. Kevin Foskett, and C. White, “Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling,” *J. Biol. Chem.*, vol. 285, no. 18, pp. 13678–13684, 2010.

[70] E. S. Dremina, V. S. Sharov, K. Kumar, A. Zaidi, E. K. Michaelis, and C. Schöneich, “Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA),” *Biochem. J.*, vol. 383, pp. 361–370, 2004.

[71] M. Chami, A. Prandini, M. Campanella, P. Pinton, G. Szabadkai, J. C. Reed, and R. Rizzuto, “Bcl-2 and bax exert opposing effects on Ca^{2+} signaling, which do not depend on their putative pore-forming region,” *J. Biol. Chem.*, vol. 279, no. 52, pp. 54581–54589, 2004.

[72] N. Erin, S. K. Bronson, and M. L. Billingsley, “Calcium-dependent interaction of calcineurin with Bcl-2 in neuronal tissue,” *Neuroscience*, vol. 117, no. 3, pp. 541–555, 2003.

[73] L. Xu, D. Kong, L. Zhu, W. Zhu, D. W. Andrews, and T. H. Kuo, “Suppression of IP_3-mediated calcium release and apoptosis by Bcl-2 involves the participation of protein phosphatase 1,” *Mol. Cell. Biochem.*, vol. 295, no. 1–2, pp. 153–165, 2007.

[74] M. J. Chang, F. Zhong, A. R. Lavik, J. B. Parys, M. J. Berridge, and C. W. Distelhorst, “Feedback regulation mediated by Bcl-2 and DARPP-32 regulates inositol 1,4,5-trisphosphate receptor phosphorylation and promotes cell survival,” *Proc. Natl. Acad. Sci.*, vol. 111, no. 3, pp. 1186–1191, 2014.

[75] Y. Wei, S. Sinha, and B. Levine, "Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation," *Autophagy*, vol. 4, no. 7, pp. 949–951, 2008.

[76] C. White, C. Li, J. Yang, N. B. Petrenko, M. Madesh, C. B. Thompson, and J. K. Foskett, "The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the $InsP_3R$," *Nat. Cell Biol.*, vol. 7, no. 10, pp. 1021–1028, 2005.

[77] C. Li, X. Wang, H. Vais, C. B. Thompson, J. K. Foskett, and C. White, "Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 104, no. 30, pp. 12565–12570, 2007.

[78] E. S. Dremina, V. S. Sharov, and C. Schöneich, "Heat-shock proteins attenuate SERCA inactivity by the anti-apoptotic protein Bcl-2: possible implications for the ER Ca^{2+}-Mediated Apoptosis," *Biochem. J.*, vol. 444, no. 1, pp. 127–139, 2012.

[79] M. Lam, G. Dubyak, L. Chen, G. Nuñez, R. L. Miesfeld, and C. W. Distelhorst, "Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 91, no. 14, pp. 6569–6573, 1994.

[80] R. Chen, I. Valencia, F. Zhong, K. S. McColl, H. L. Roderick, M. D. Bootman, M. J. Berridge, S. J. Conway, A. B. Holmes, G. A. Mignery, P. Velez, and C. W. Distelhorst, "Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate," *J. Cell Biol.*, vol. 166, no. 2, pp. 193–203, 2004.

[81] C. J. Hanson, M. D. Bootman, C. W. Distelhorst, R. J. H. Wojcikiewicz, and H. L. Roderick, "Bcl-2 suppresses Ca^{2+} release through inositol 1,4,5-trisphosphate receptors and inhibits Ca^{2+} uptake by mitochondria without affecting ER calcium store content," *Cell Calcium*, vol. 44, no. 3, pp. 324–338, 2008.

[82] F. Zhong, M. C. Davis, K. S. McColl, and C. W. Distelhorst, "Bcl-2 differentially regulates Ca^{2+} signals according to the strength of T cell receptor activation," *J. Cell Biol.*, vol. 172, no. 1, pp. 127–137, 2006.

[83] Y. Rong, G. Bultynck, A. S. Aromolaran, F. Zhong, J. B. Parys, H. De Smedt, G. A. Mignery, H. L. Roderick, M. D. Bootman, and C. W. Distelhorst, "The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP_3 receptor," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 34, pp. 14397–14402, 2009.

[84] G. Monaco, E. Decrock, H. Akl, R. Ponsaerts, T. Vervliet, T. Luyten, M. De Maeyer, L. Missiaen, C. W. Distelhorst, H. De Smedt, J. B. Parys, L. Leybaert, and G. Bultynck, "Selective regulation of IP_3-receptor-mediated Ca^{2+} signaling and apoptosis by the BH4 domain of Bcl-2 versus Bcl-Xl," *Cell Death Differ.*, vol. 19, no. 2, pp. 295–309, 2012.

[85] G. Monaco, E. Decrock, K. Nuyts, L. E. Wagner, T. Luyten, S. V. Strelkov, L. Missiaen, W. M. De Borggraeve, L. Leybaert, D. I. Yule, H. De Smedt, J. B. Parys, and G. Bultynck, "Alpha-helical destabilization of the Bcl-2-BH4-domain peptide abolishes its ability to inhibit the IP_3 receptor," *PLoS One*, vol. 8, no. 8: e73386, 2013.

[86] Y. Rong, A. S. Aromolaran, G. Bultynck, F. Zhong, X. Li, K. McColl, S. Matsuyama, S. Herlitze, H. L. Roderick, M. D. Bootman, G. A. Mignery, J. B. Parys, H. De Smedt, and C. W. Distelhorst, "Targeting Bcl-2-IP_3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals," *Mol. Cell*, vol. 31, no. 2, pp. 255–265, 2008.

[87] F. Zhong, M. W. Harr, G. Bultynck, J. B. Parys, H. De Smedt, Y. Rong, J. K. Molitoris, M. Lam, C. Ryder, S. Matsuyama, and C. W. Distelhorst, "Induction of Ca^{2+}-driven apoptosis in chronic lymphocytic leukemia cells by peptide-mediated disruption of Bcl-2-IP_3 receptor interaction," *Blood*, vol. 117, no. 10, pp. 2924–2934, 2011.

[88] H. Akl, G. Monaco, R. La Rovere, K. Welkenhuyzen, S. Kiviluoto, T. Vervliet, J. Molgó, C. W. Distelhorst, L. Missiaen, K. Mikoshiba, J. B. Parys, H. De Smedt, and G. Bultynck, "IP_3R2 levels dictate the apoptotic sensitivity of diffuse large B-cell lymphoma cells to an IP_3R-derived peptide targeting the BH4 domain of Bcl-2.," *Cell Death Dis.*, vol. 4: e632, 2013.

[89] A. Lavik, F. Zhong, M. Chang, E. Greenberg, Y. Choudhary, M. Smith, K. McColl, J. Pink, F. Reu, S. Matsuyama, and C. Distelhorst, "A synthetic peptide targeting the BH4 domain of Bcl-2 induces apoptosis in multiple myeloma and follicular lymphoma cells alone or in combination

with agents targeting the BH3-binding pocket of Bcl-2," *Oncotarget*, vol. 6, no. 29, pp. 27388-27402, 2015.

[90] E. F. Greenberg, K. S. McColl, F. Zhong, G. Wildey, A. Dowlati, and C. W. Distelhorst, "Synergistic killing of human small cell lung cancer cells by the Bcl-2-inositol 1,4,5-trisphosphate receptor disruptor BIRD-2 and the BH3-mimetic ABT-263," *Cell Death Dis.*, vol. 6: e2034, 2015.

[91] H. Akl, R. M. L. La Rovere, A. Janssens, P. Vandenberghe, J. B. Parys, and G. Bultynck, "HA14-1 potentiates apoptosis in B-cell cancer cells sensitive to a peptide disrupting IP_3 receptor/Bcl-2 complexes," *Int. J. Dev. Biol.*, 2015.

[92] C. W. Distelhorst and G. C. Shore, "Bcl-2 and calcium: controversy beneath the surface," *Oncogene*, vol. 23, no. 16, pp. 2875–2880, 2004.

[93] Q. Xu and J. C. Reed, "Bax inhibitor-1, a mammalian apoptosis suppressor identified by functional screening in yeast," *Mol. Cell*, vol. 1, no. 3, pp. 337–346, 1998.

[94] G. Bultynck, S. Kiviluoto, N. Henke, H. Ivanova, L. Schneider, V. Rybalchenko, T. Luyten, K. Nuyts, W. De Borggraeve, I. Bezprozvanny, J. B. Parys, H. De Smedt, L. Missiaen, and A. Methner, "The C terminus of bax inhibitor-1 forms a Ca^{2+}-permeable channel pore," *J. Biol. Chem.*, vol. 287, no. 4, pp. 2544–2557, 2012.

[95] G. Bultynck, S. Kiviluoto, and A. Methner, "Bax inhibitor-1 is likely a pH-sensitive calcium leak channel, not a H^+/Ca^{2+} exchanger," *Sci. Signal.*, vol. 7, no. 343, 2014.

[96] D. Rojas-Rivera and C. Hetz, "TMBIM protein family: ancestral regulators of cell death," *Oncogene*, vol. 34, no. 3, pp. 269–280, 2015.

[97] C. Xu, W. Xu, A. E. Palmer, and J. C. Reed, "BI-1 regulates endoplasmic reticulum Ca^{2+} homeostasis downstream of Bcl-2 family proteins," *J. Biol. Chem.*, vol. 283, no. 17, pp. 11477–11484, 2008.

[98] N. S. Wang, M. T. Unkila, E. Z. Reineks, and C. W. Distelhorst, "Transient expression of wild-type or mitochondrially targeted Bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted Bcl-2 is protective against Bax-induced cell death," *J. Biol. Chem.*, vol. 276, no. 47, pp. 44117–44128, 2001.

[99] C. J. Hanson, M. D. Bootman, C. W. Distelhorst, T. Maraldi, and H. L. Roderick, "The cellular concentration of Bcl-2 determines its pro- or anti-apoptotic effect," *Cell Calcium*, vol. 44, no. 3, pp. 243–258, 2008.

[100] T. Vervliet, E. Decrock, J. Molgó, V. Sorrentino, L. Missiaen, L. Leybaert, H. De Smedt, N. N. Kasri, J. B. Parys, and G. Bultynck, "Bcl-2 binds to and inhibits ryanodine receptors," *J. Cell Sci.*, vol. 127, pp. 2782–2792, 2014.

[101] T. Vervliet, J. B. Parys, and G. Bultynck, "Bcl-2 and FKBP12 bind to IP_3 and ryanodine receptors at overlapping sites: the complexity of protein-protein interactions for channel regulation.," *Biochem. Soc. Trans.*, vol. 43, no. 3, pp. 396–404, 2015.

[102] T. Vervliet, I. Lemmens, K. Welkenhuyzen, J. Tavernier, J. B. Parys, and G. Bultynck, "Regulation of the ryanodine receptor by anti-apoptotic Bcl-2 is independent of its BH3-domain-binding properties," *Biochem. Biophys. Res. Commun.*, vol. 463, no. 3, pp. 174–179, 2015.

[103] L. Zhang, Y. Liu, F. Song, H. Zheng, L. Hu, H. Lu, P. Liu, X. Hao, W. Zhang, and K. Chen, "Functional SNP in the microRNA-367 binding site in the 3'UTR of the calcium channel ryanodine receptor gene 3 (RYR3) affects breast cancer risk and calcification," *Proc. Natl. Acad. Sci.*, vol. 108, no. 33, pp. 13653–13658, 2011.

[104] F. M. Davis, M. T. Parsonage, P. J. Cabot, M. O. Parat, E. W. Thompson, S. J. Roberts-Thomson, and G. R. Monteith, "Assessment of gene expression of intracellular calcium channels, pumps and exchangers with epidermal growth factor-induced epithelial-mesenchymal transition in a breast cancer cell line," *Cancer Cell Int.*, vol. 13, no. 1, pp. 76–83, 2013.

[105] L. H. Boise, M. González-García, C. E. Postema, L. Ding, T. Lindsten, L. A. Turka, X. Mao, G. Nuñez, and C. B. Thompson, "Bcl-X, a Bcl-2-related gene that functions as a dominant regulator of apoptotic cell death," *Cell*, vol. 74, no. 4, pp. 597–608, 1993.

[106] S. Thomas, B. A. Quinn, S. K. Das, R. Dash, L. Emdad, S. Dasgupta, X. Y. Wang, P. Dent, J. C. Reed, M. Pellecchia, D. Sarkar, and P. B. Fisher, "Targeting the Bcl-2 family for cancer therapy," *Expert Opin Ther Targets*, vol. 17, no. 1, pp. 61–75, 2013.

[107] K. Zhang, K. Jiao, Z. Xing, L. Zhang, J. Yang, X. Xie, and L. Yang, "Bcl-xL overexpression and its association with the progress of tongue carcinoma," *Int. J. Clin. Exp. Pathol.*, vol. 7, no. 11, pp. 7360–7377, 2014.

[108] M. Nagane, A. Levitzki, A. Gazit, W. K. Cavenee, and H. J. Huang, "Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 95, no. 10, pp. 5724–5729, 1998.

[109] K. D. Mason, M. R. Carpinelli, J. I. Fletcher, J. E. Collinge, A. A. Hilton, S. Ellis, P. N. Kelly, P. G. Ekert, D. Metcalf, A. W. Roberts, D. C. S. Huang, and B. T. Kile, "Programmed anuclear cell death delimits platelet life span," *Cell*, vol. 128, no. 6, pp. 1173–1186, 2007.

[110] J. D. Leverson, D. C. Phillips, M. J. Mitten, E. R. Boghaert, D. Diaz, S. K. Tahir, L. D. Belmont, P. Nimmer, Y. Xiao, X. M. Ma, K. N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T. J. Magoc, K. S. Vaidya, D. H. Albert, J. M. Tarrant, N. La, L. Wang, Z. F. Tao, M. D. Wendt, D. Sampath, S. H. Rosenberg, C. Tse, D. C. S. Huang, W. J. Fairbrother, S. W. Elmore, and A. J. Souers, "Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy," *Sci. Transl. Med.*, vol. 7, no. 279: 279ra40, 2015.

[111] J. Foskett, J. Yang, K. Cheung, and H. Vais, "Bcl-xL regulation of $InsP_3$ receptor gating mediated by dual Ca^{2+} release channel BH3 domains," *Biophys. J.*, vol. 96, pp. 391–392, 2009.

[112] T. Vervliet, I. Lemmens, E. Vandermarliere, E. Decrock, H. Ivanova, G. Monaco, V. Sorrentino, N. N. Kasri, L. Missiaen, L. Martens, H. De Smedt, L. Leybaert, J. B. Parys, J. Tavernier, and G. Bultynck, "Ryanodine receptors are targeted by anti-apoptotic Bcl-XL involving its BH4 domain and Lys87 from its BH3 domain," *Sci. Rep.*, vol. 5, p. 9641, 2015.

[113] S. Shimizu, A. Konishi, T. Kodama, and Y. Tsujimoto, "BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 7, pp. 3100–3105, 2000.

[114] H. Huang, X. Hu, C. O. Eno, G. Zhao, C. Li, and C. White, "An interaction between Bcl-xL and the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca^{2+} uptake," *J. Biol. Chem.*, vol. 288, no. 27, pp. 19870–19881, 2013.

[115] N. Arbel, D. Ben-Hail, and V. Shoshan-Barmatz, "Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein," *J. Biol. Chem.*, vol. 287, no. 27, pp. 23152–23161, 2012.

[116] G. Monaco, E. Decrock, N. Arbel, A. R. van Vliet, R. La Rovere, H. De Smedt, J. B. Parys, P. Agostinis, L. Leybaert, V. Shoshan-Barmatz, and G. Bultynck, "The BH4 domain of anti-apoptotic Bcl-XL, but not that of the related Bcl-2, limits the voltage-dependent anion channel 1 (VDAC1)-mediated transfer of pro-apoptotic Ca^{2+} signals to mitochondria," *J. Biol. Chem.*, vol. 290, no. 14, pp. 9150–9161, 2015.

[117] V. Shoshan-Barmatz, D. Ben-Hail, L. Admoni, Y. Krelin, and S. S. Tripathi, "The mitochondrial voltage-dependent anion channel 1 in tumor cells," *Biochim. Biophys. Acta*, vol. 1848, no. 10, pp. 2547–2575, 2015.

[118] J. T. Opferman, A. Letai, C. Beard, M. D. Sorcinelli, C. C. Ong, and S. J. Korsmeyer, "Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1," *Nature*, vol. 426, pp. 671–676, 2003.

[119] I. Dzhagalov, A. Dunkle, and Y. W. He, "The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages," *J. Immunol.*, vol. 181, no. 1, pp. 521–528, 2008.

[120] N. Arbour, J. L. Vanderluit, J. N. Le Grand, A. Jahani-Asl, V. A. Ruzhynsky, E. C. C. Cheung, M. A. Kelly, A. E. MacKenzie, D. S. Park, J. T. Opferman, and R. S. Slack, "Mcl-1 is a key regulator of

apoptosis during CNS development and after DNA damage," *J. Neurosci.*, vol. 28, no. 24, pp. 6068–6078, 2008.

[121] J. T. Opferman, H. Iwasaki, C. C. Ong, H. Suh, S. Mizuno, K. Akashi, and S. J. Korsmeyer, "Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells," *Science*, vol. 307, pp. 1101–1104, 2005.

[122] J. L. Rinkenberger, S. Horning, B. Klocke, K. Roth, and S. J. Korsmeyer, "Mcl-1 deficiency results in peri-implantation embryonic lethality," *Genes Dev.*, vol. 14, no. 1, pp. 23–27, 2000.

[123] C. Akgul, "Mcl-1 is a potential therapeutic target in multiple types of cancer," *Cell. Mol. Life Sci.*, vol. 66, no. 8, pp. 1326–1336, 2009.

[124] R. Beroukhim, C. H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J. S. Boehm, J. Dobson, M. Urashima, K. T. Mc Henry, R. M. Pinchback, A. H. Ligon, Y. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M. S. Lawrence, B. A. Weir, K. E. Tanaka, D. Y. Chiang, A. J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F. J. Kaye, H. Sasaki, J. E. Tepper, J. A. Fletcher, J. Tabernero, J. Baselga, M. S. Tsao, F. Demichelis, M. A. Rubin, P. A. Janne, M. J. Daly, C. Nucera, R. L. Levine, B. L. Ebert, S. Gabriel, A. K. Rustgi, C. R. Antonescu, M. Ladanyi, A. Letai, L. A. Garraway, M. Loda, D. G. Beer, L. D. True, A. Okamoto, S. L. Pomeroy, S. Singer, T. R. Golub, E. S. Lander, G. Getz, W. R. Sellers, and M. Meyerson, "The landscape of somatic copy-number alteration across human cancers.," *Nature*, vol. 463, no. 7283, pp. 899–905, 2010.

[125] J. Belmar and S. W. Fesik, "Small molecule Mcl-1 inhibitors for the treatment of cancer," *Pharmacol. Ther.*, vol. 145, pp. 76–84, 2015.

[126] H. Huang, K. Shah, N. A. Bradbury, C. Li, and C. White, "Mcl-1 promotes lung cancer cell migration by directly interacting with VDAC to increase mitochondrial Ca^{2+} uptake and reactive oxygen species generation," *Cell Death Dis.*, vol. 5: e1482, 2014.

[127] G. Morciano, C. Giorgi, D. Balestra, S. Marchi, M. Pinotti, and P. Pinton, "Mcl-1 involvement in mitochondrial dynamics is associated with apoptotic cell death," *Mol. Biol. Cell*, vol. 27, pp. 20–34, 2016.

[128] S. Y. Hsu, A. Kaipia, E. McGee, M. Lomeli, and A. J. Hsueh, "Bok is a pro-apoptotic Bcl-2 protein with restricted expression in reproductive tissues and heterodimerizes with selective anti-apoptotic Bcl-2 family members," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, no. 23, pp. 12401–12406, 1997.

[129] F. Ke, A. Voss, J. B. Kerr, L. O'Reilly, L. Tai, N. Echeverry, P. Bouillet, A. Strasser, and T. Kaufmann, "BCL-2 family member BOK is widely expressed but its loss has only minimal impact in mice," *Cell Death Differ.*, vol. 19, no. 6, pp. 915–925, 2012.

[130] N. Echeverry, D. Bachmann, F. Ke, A. Strasser, H. U. Simon, and T. Kaufmann, "Intracellular localization of the BCL-2 family member BOK and functional implications," *Cell Death Differ.*, vol. 20, no. 6, pp. 785–799, 2013.

[131] J. Lippincott-Schwartz, L. C. Yuan, J. S. Bonifacino, and R. D. Klausner, "Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER," *Cell*, vol. 56, no. 5, pp. 801–813, 1989.

[132] M. A. Carpio, M. Michaud, W. Zhou, J. K. Fisher, L. D. Walensky, and S. G. Katz, "BCL-2 family member BOK promotes apoptosis in response to endoplasmic reticulum stress," *Proc. Natl. Acad. Sci.*, vol. 112, no. 23, pp. 7201–7206, 2015.

[133] J. J. Schulman, F. A. Wright, T. Kaufmann, and R. J. H. Wojcikiewicz, "The Bcl-2 protein family member bok binds to the coupling domain of inositol 1,4,5-trisphosphate receptors and protects them from proteolytic cleavage," *J. Biol. Chem.*, vol. 288, no. 35, pp. 25340–25349, 2013.

[134] J. Hirota, T. Furuichi, and K. Mikoshiba, "Inositol 1,4,5-trisphosphate receptor type 1 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent manner," *J. Biol. Chem.*, vol. 274, no. 48, pp. 34433–34437, 1999.

[135] Z. Assefa, G. Bultynck, K. Szlufcik, N. Nadif Kasri, E. Vermassen, J. Goris, L. Missiaen, G. Callewaert, J. B. Parys, and H. De Smedt, "Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis," *J. Biol. Chem.*, vol. 279, no. 41, pp. 43227–43236, 2004.

[136] L. Verbert, B. Lee, S. Kocks, Z. Assefa, J. Parys, L. Missiaen, G. Callewaert, R. Fissore, H. De Smedt, and G. Bultynck, "Caspase-3-truncated type 1 inositol 1,4,5-trisphosphate receptor enhances intracellular Ca^{2+} leak and disturbs Ca^{2+} signalling," *Biol. Cell*, vol. 100, no. 1, pp. 39–49, 2008.

[137] N. Zhang and R. A. Fissore, "Role of caspase-3 cleaved IP_3R1 on Ca^{2+} homeostasis and developmental competence of mouse oocytes and eggs," *J. Cell. Physiol.*, vol. 229, no. 11, pp. 1842–1854, 2014.

[138] G. Elkoreh, V. Blais, É. Béliveau, G. Guillemette, and J. B. Denault, "Type 1 inositol-1,4,5-trisphosphate receptor is a late substrate of caspases during apoptosis," *J. Cell. Biochem.*, vol. 113, no. 8, pp. 2775–2784, 2012.

[139] A. M. Akimzhanov, J. M. Barral, and D. Boehning, "Caspase 3 cleavage of the inositol 1,4,5-trisphosphate receptor does not contribute to apoptotic calcium release," *Cell Calcium*, vol. 53, no. 2, pp. 152–158, 2013.

[140] L. L. Fu, Y. Cheng, and B. Liu, "Beclin-1: Autophagic regulator and therapeutic target in cancer," *Int. J. Biochem. Cell Biol.*, vol. 45, no. 5, pp. 921–924, 2013.

[141] E. Toton, N. Lisiak, P. Sawicka, and M. Rybczynska, "Beclin-1 and its role as a target for anticancer therapy," *J. Physiol. Pharmacol.*, vol. 64, no. 4, pp. 459–467, 2014.

[142] R. Kang, H. J. Zeh, M. T. Lotze, and D. Tang, "The Beclin 1 network regulates autophagy and apoptosis," *Cell Death Differ.*, vol. 18, no. 4, pp. 571–580, 2011.

[143] J. P. Decuypere, J. B. Parys, and G. Bultynck, "Regulation of the autophagic bcl-2/beclin 1 interaction," *Cells*, vol. 1, no. 3, pp. 284–312, 2012.

[144] S. Turcotte and A. J. Giaccia, "Targeting cancer cells through autophagy for anticancer therapy," *Curr. Opin. Cell Biol.*, vol. 22, no. 2, pp. 246–251, 2010.

[145] J. P. Decuypere, K. Welkenhuyzen, T. Luyten, R. Ponsaerts, M. Dewaele, J. Molgó, P. Agostinis, L. Missiaen, H. De Smedt, J. B. Parys, and G. Bultynck, "$Ins(1,4,5)P_3$ receptor-mediated Ca^{2+} signaling and autophagy induction are interrelated," *Autophagy*, vol. 7, no. 12, pp. 1472–1489, 2011.

[146] J. M. Vicencio, C. Ortiz, A. Criollo, A. W. E. Jones, O. Kepp, L. Galluzzi, N. Joza, I. Vitale, E. Morselli, M. Tailler, M. Castedo, M. C. Maiuri, J. Molgó, G. Szabadkai, S. Lavandero, and G. Kroemer, "The inositol 1,4,5-trisphosphate receptor regulates autophagy through its interaction with Beclin 1," *Cell Death Differ.*, vol. 16, no. 7, pp. 1006–1017, 2009.

[147] B. A. Hemmings and D. F. Restuccia, "PI3K-PKB/Akt pathway," *Cold Spring Harb. Perspect. Biol.*, vol. 4, no. 9: a011189, 2012.

[148] C. A. Worby and J. E. Dixon, "PTEN," *Annu. Rev. Biochem.*, vol. 83, no. 1, pp. 641–669, 2014.

[149] M. Milella, I. Falcone, F. Conciatori, U. Cesta Incani, A. Del Curatolo, N. Inzerilli, C. M. A. Nuzzo, V. Vaccaro, S. Vari, F. Cognetti, and L. Ciuffreda, "PTEN: multiple functions in human malignant tumors," *Front. Oncol.*, vol. 5, pp. 1–14, 2015.

[150] M. Martini, M. C. De Santis, L. Braccini, F. Gulluni, and E. Hirsch, "PI3K/AKT signaling pathway and cancer: an updated review," *Ann. Med.*, vol. 34, pp. 1–12, 2014.

[151] M. T. Khan, L. Wagner, D. I. Yule, C. Bhanumathy, and S. K. Joseph, "Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors," *J. Biol. Chem.*, vol. 281, no. 6, pp. 3731–3737, 2006.

[152] T. Szado, V. Vanderheyden, J. B. Parys, H. De Smedt, K. Rietdorf, L. Kotelevets, E. Chastre, F. Khan, U. Landegren, O. Söderberg, M. D. Bootman, and H. L. Roderick, "Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca^{2+} release and apoptosis," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 7, pp. 2427–2432, 2008.

[153] S. Marchi, A. Rimessi, C. Giorgi, C. Baldini, L. Ferroni, R. Rizzuto, and P. Pinton, "Akt kinase reducing endoplasmic reticulum Ca^{2+} release protects cells from Ca^{2+}-dependent apoptotic stimuli," *Biochem. Biophys. Res. Commun.*, vol. 375, no. 4, pp. 501–505, 2008.

[154] S. Marchi, M. Marinello, A. Bononi, M. Bonora, C. Giorgi, A. Rimessi, and P. Pinton, "Selective modulation of subtype III IP_3R by Akt regulates ER Ca^{2+} release and apoptosis," *Cell Death Dis.*, vol. 3, no. 5: e304, 2012.

[155] R. S. Hotchkiss, A. Strasser, J. E. Mcdunn, and P. E. Swanson, "Type 3 inositol 1,4,5-trisphosphate receptor modulates cell death," *FASEB J.*, vol. 14, no. 10, pp. 1375–1379, 2000.

[156] C. C. P. Mendes, D. A. Gomes, M. Thompson, N. C. Souto, T. S. Goes, A. M. Goes, M. A. Rodrigues, M. V Gomez, M. H. Nathanson, and M. F. Leite, "The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca^{2+} signals into mitochondria," *J. Biol. Chem.*, vol. 280, no. 49, pp. 40892–40900, 2005.

[157] T. Jayaraman and A. R. Marks, "T cells deficient in inositol 1,4,5-trisphosphate receptor are resistant to apoptosis," *Mol. Cell. Biol.*, vol. 17, no. 6, pp. 3005–3012, 1997.

[158] A. Bononi, M. Bonora, S. Marchi, S. Missiroli, F. Poletti, C. Giorgi, P. P. Pandolfi, and P. Pinton, "Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis in a protein phosphatase-dependent manner," *Cell Death Differ*, vol. 20, no. 12, pp. 1631–1643, 2013.

[159] P. Salomoni and P. P. Pandolfi, "The role of PML in tumor suppression," *Cell*, vol. 108, pp. 165–170, 2002.

[160] P. Salomoni, B. Ferguson, A. Wyllie, and T. Rich, "New insights into the role of PML in tumour suppression," *Cell Res.*, vol. 18, pp. 622–640, 2008.

[161] P. Pinton, C. Giorgi, and P. P. Pandolfi, "The role of PML in the control of apoptotic cell fate: a new key player at ER–mitochondria sites," *Cell Death Differ.*, vol. 18, pp. 1450–1456, 2011.

[162] R. Bernardi, A. Papa, and P. P. Pandolfi, "Regulation of apoptosis by PML and the PML-NBs," *Oncogene*, vol. 27, pp. 6299–6312, 2008.

[163] C. Giorgi, K. Ito, H. Lin, C. Santangelo, M. R. Wieckowski, M. Lebiedzinska, A. Bononi, M. Bonora, J. Duszynski, R. Bernardi, R. Rizzuto, C. Tacchetti, P. Pinton, and P. P. Pandolfi, "PML regulates apoptosis at endoplasmic reticulum by modulating calcium release," *Science*, vol. 330, pp. 1247–1251, 2010.

[164] D. P. Silver and D. M. Livingston, "Mechanisms of BRCA1 tumor suppression," *Cancer Discov.*, vol. 2, pp. 679–684, 2012.

[165] A. E. Elia and S. J. Elledge, "BRCA1 as tumor suppressor: lord without its RING?," *Breast Cancer Res.*, vol. 14, no. 2, p. 306, 2012.

[166] N. Makharashvili and T. T. Paull, "CtIP: A DNA damage response protein at the intersection of DNA metabolism," *DNA Repair (Amst).*, vol. 32, pp. 75–81, 2015.

[167] A. Castillo, A. Paul, B. Sun, T. H. Huang, Y. Wang, S. A. Yazinski, J. Tyler, L. Li, M. J. You, L. Zou, J. Yao, and B. Wang, "The BRCA1-interacting protein Abraxas is required for genomic stability and tumor suppression," *Cell Rep.*, vol. 8, no. 3, pp. 807–817, 2014.

[168] S. B. Cantor and S. Guillemette, "Hereditary breast cancer and the BRCA1-associated FANCJ/BACH1/BRIP1," *Futur. Oncol.*, vol. 7, no. 2, pp. 253–261, 2011.

[169] S. C. Hedgepeth, M. I. Garcia, L. E. Wagner, A. M. Rodriguez, S. V. Chintapalli, R. R. Snyder, G. D. V. Hankins, B. R. Henderson, K. M. Brodie, D. I. Yule, D. B. van Rossum, and D. Boehning, "The BRCA1 tumor suppressor binds to inositol 1,4,5-trisphosphate receptors to stimulate apoptotic calcium release," *J. Biol. Chem.*, vol. 290, no. 11, pp. 7304–7313, 2015.

[170] J. Liu, C. Zhang, and Z. Feng, "Tumor suppressor p53 and its gain-of-function mutants in cancer," *Acta Biochim. Biophys. Sin. (Shanghai).*, vol. 46, no. 3, pp. 170–179, 2014.

[171] J. Liu, C. Zhang, W. Hu, and Z. Feng, "Tumor suppressor p53 and its mutants in cancer metabolism," *Cancer Lett.*, vol. 356, no. 2, pp. 197–203, 2015.

[172] M. J. Duffy, N. C. Synnott, P. M. McGowan, J. Crown, D. O'Connor, and W. M. Gallagher, "P53 as a target for the treatment of cancer," *Cancer Treat. Rev.*, vol. 40, no. 10, pp. 1153–1160, 2014.

[173] C. Giorgi, M. Bonora, G. Sorrentino, S. Missiroli, F. Poletti, J. M. Suski, F. Galindo Ramirez, R. Rizzuto, F. Di Virgilio, E. Zito, P. P. Pandolfi, M. R. Wieckowski, F. Mammano, G. Del Sal, and P. Pinton, "p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+}-dependent manner," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 6, pp. 2–7, 2015.

[174] C. Giorgi, M. Bonora, and P. Pinton, "Inside the tumor: p53 modulates calcium homeostasis," *Cell Cycle*, vol. 14, no. 7, pp. 933–934, 2015.

[175] C. Giorgi, M. Bonora, S. Missiroli, F. Poletti, F. G. Ramirez, G. Morciano, C. Morganti, P. P. Pandolfi, F. Mammano, and P. Pinton, "Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling," *Oncotarget*, vol. 6, no. 3, pp. 1435–1445, 2015.

[176] M. Bonora, C. Giorgi, and P. Pinton, "Novel frontiers in calcium signaling: A possible target for chemotherapy," *Pharmacol. Res.*, vol. 99, pp. 82–85, 2015.

[177] M. Bittremieux and G. Bultynck, "p53 and Ca^{2+} signaling from the endoplasmic reticulum: partners in anti-cancer therapies," *Oncoscience*, vol. 2, no. 3, pp. 233–238, 2015.

[178] A. E. Karnoub and R. A. Weinberg, "Ras oncogenes: split personalities," *Nat. Rev. Mol. Cell Biol.*, vol. 9, pp. 517–531, 2008.

[179] S. Schubbert, K. Shannon, and G. Bollag, "Hyperactive Ras in developmental disorders and cancer," *Nat. Rev. Cancer*, vol. 7, pp. 295–308, 2007.

[180] E. Schmukler, Y. Kloog, and R. P. Kramarski, "Ras and autophagy in cancer development and therapy," *Oncotarget*, vol. 5, no. 3, pp. 577–586, 2014.

[181] M. Hashii, Y. Nozawa, and H. Higashida, "Bradykinin-induced cytosolic Ca^{2+} oscillations and inositol tetrakisphosphate-induced Ca^{2+} influx in voltage-clamped ras-transformed NIH/3T3 fibroblasts," *J. Biol. Chem.*, vol. 268, no. 26, pp. 19403–19410, 1993.

[182] C. Song, C. D. Hu, M. Masago, K. I. Kariya, Y. Yamawaki-Kataoka, M. Shibatohge, D. Wu, T. Satoh, and T. Kataoka, "Regulation of a novel human phospholipase C, PLCepsilon, through membrane targeting by Ras," *J. Biol. Chem.*, vol. 276, no. 4, pp. 2752–2757, 2001.

[183] T. D. Bunney, R. Harris, N. L. Gandarillas, M. B. Josephs, S. M. Roe, S. C. Sorli, H. F. Paterson, F. Rodrigues-Lima, D. Esposito, C. P. Ponting, P. Gierschik, L. H. Pearl, P. C. Driscoll, and M. Katan, "Structural and mechanistic insights into ras association domains of phospholipase C epsilon," *Mol. Cell*, vol. 21, no. 4, pp. 495–507, 2006.

[184] C. Pierro, S. J. Cook, T. C. Foets, M. D. Bootman, and H. L. Roderick, "Oncogenic K-Ras suppresses IP_3-dependent Ca^{2+} release through remodelling of the isoform composition of IP_3Rs and ER luminal Ca^{2+} levels in colorectal cancer cell lines," *J Cell Sci*, vol. 127, pp. 1607–1619, 2014.

[185] T. G. Bivona, S. E. Quatela, B. O. Bodemann, I. M. Ahearn, M. J. Soskis, A. Mor, J. Miura, H. H. Wiener, L. Wright, S. G. Saba, D. Yim, A. Fein, I. Pérez de Castro, C. Li, C. B. Thompson, A. D. Cox, and M. R. Philips, "PKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis," *Mol. Cell*, vol. 21, no. 4, pp. 481–493, 2006.

[186] P. J. Sung, F. D. Tsai, H. Vais, H. Court, J. Yang, N. Fehrenbacher, J. K. Foskett, and M. R. Philips, "Phosphorylated K-Ras limits cell survival by blocking Bcl-xL sensitization of inositol trisphosphate receptors," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 51, pp. 20593–20598, 2013.

[187] A. Rimessi, S. Marchi, S. Patergnani, and P. Pinton, "H-Ras-driven tumoral maintenance is sustained through caveolin-1-dependent alterations in calcium signaling," *Oncogene*, vol. 33, no. 18, pp. 2329–2340, 2014.

Legends figures

Fig. 1: ER-mitochondrial Ca^{2+} signaling in healthy cells and in cancer cells. Oncogenes are indicated in green, whereas tumor suppressors are depicted in red. Bar-headed lines indicate an inhibitory interaction. Arrow-headed lines indicate a stimulatory interaction. The black arrows represent Ca^{2+} fluxes. The level of shading of the ER corresponds to the ER [Ca^{2+}], i.e. a darker shade indicates a higher [Ca^{2+}] while lighter shades represent lower Ca^{2+} levels. *Left Panel,* In normal, healthy cells, tumor suppressors (PTEN, BRCA1, Beclin 1, and p53) and oncogenes (Bcl-2-family members, PKB/Akt, and Ras) are in balance, thereby providing proper Ca^{2+} transfer from the ER to the mitochondria. This Ca^{2+} flux ensures cell survival by driving mitochondrial bioenergetics. SERCA pumps cytosolic Ca^{2+} into the ER lumen, whereas Ca^{2+} is released from the ER by the IP_3R. Ca^{2+} released from the ER is taken up by the mitochondria via VDAC1, present in the outer mitochondrial membrane, and MCU, located in the inner mitochondrial membrane. *Right Panel,* Alterations to the ER-mitochondrial Ca^{2+}-signaling pathway can render cancer cells resistant to cell death stimuli. Upregulation of oncogenes, such as Bcl-2, and/or downregulation of tumor suppressors, like p53, prevent the occurrence of toxic pro-apoptotic ER-mitochondrial Ca^{2+} fluxes.

Fig. 2: Indirect modulation of the Ca^{2+}-mediated cell death response in damaged healthy or damaged cancer cells by tumor suppressors and oncogenes. Symbols and colors are as explained in the legend of figure 1. *Left Panel,* Pro-apoptotic ER-mitochondrial Ca^{2+} signaling events can be modulated indirectly by altering the ER Ca^{2+}-store content. Tumor suppressor p53 is upregulated in healthy cells that became damaged due to various stress conditions, such as the presence of toxic compounds or DNA damage. Upregulation of p53, which stimulates SERCA activity and therefore results in an increase of the ER Ca^{2+} content, boosts excessive Ca^{2+} transfers from the ER to the mitochondria and subsequently leads to mitochondrial Ca^{2+} overload and apoptotic cell death. *Right Panel,* Ca^{2+}-mediated cell death can be circumvented in stressed cancer cells by a decreased ER Ca^{2+} content, which indirectly prevents mitochondrial Ca^{2+} overload. Oncogenes Bcl-2 and Ras can decrease the ER store content by inhibiting the SERCA pump or by lowering SERCA2b expression levels respectively. Bcl-XL, Mcl-1 and Bcl-2 can also sensitize IP_3Rs located in parts of the ER membrane outside of the MAMs to low levels of IP_3 signaling, promoting basal ER Ca^{2+} leak. This will lower the ER Ca^{2+} levels and prevent cell death caused by mitochondrial Ca^{2+} overload.

Fig. 3: Direct modulation of the Ca^{2+}-mediated cell death response in stressed healthy or stressed cancer cells by tumor suppressors and oncogenes. Symbols and colors are as explained in the legend of figure 1. *Left Panel,* Apoptotic cell death is stimulated in damaged healthy cells by directly modulating IP_3R-mediated Ca^{2+} signaling events. Tumor suppressors PTEN, PML and BRCA1 increase IP_3R activity, thereby facilitating mitochondrial Ca^{2+} overload. In contrast to PTEN and BRCA1, PML appears to act indirectly on the IP_3R by facilitating the recruitment of PP2A, which counteracts the apoptosis-suppressing effects of PKB/Akt on the IP_3Rs. *Right Panel,* Upregulated levels of oncogenes that reduce toxic ER-mitochondrial Ca^{2+} transfers can cause malignant cells to survive stress conditions, like stress induced by anti-cancer therapies. Bcl-2 as well as PKB/Akt suppress excessive, pro-apoptotic Ca^{2+} signaling events by directly inhibiting IP_3R activity. Bcl-XL inhibits VDAC1-mediated Ca^{2+} uptake into the mitochondria, hence preventing mitochondrial Ca^{2+} overload.

Fig. 4: Regulation of mitochondrial bioenergetics by oncogenes and tumor suppressors. Symbols and colors are as explained in the legend of figure 1. *Left Panel,* Mitochondrial energy production is

maintained by constitutive low-level IP_3R-mediated Ca^{2+} transfer from the ER to the mitochondria, boosting ATP production. Low-level IP_3R-mediated Ca^{2+} signaling is stimulated by Mcl-1, Bcl-2 and Bcl-XL, which sensitize the IP_3Rs present in the MAMs to basal IP_3 levels. Mcl-1 also increases mitochondrial Ca^{2+} uptake by stimulating VDAC1's Ca^{2+}-flux properties. *Right Panel,* Phosphorylated K-Ras4B suppresses mitochondrial Ca^{2+} dynamics by counteracting the sensitization of IP_3Rs by Bcl-XL, thereby suppressing mitochondrial metabolism and triggering autophagic cell death.

Fig. 5: Linear representation of the IP_3R isoform 1 and its regulation by tumor suppressors and oncogenes. Oncogenes are represented in green, while tumor suppressors are indicated in red. Bar-headed lines indicate an inhibitory interaction. Arrow-headed lines indicate a stimulatory interaction. Interactions with the IP_3R without functional effect are represented by oval arrows. The IP_3R1 consists of three large functional domains: a ligand-binding domain, which is composed of a suppressor domain and an IP_3-binding core, a modulatory and transducing domain, and a C-terminal channel region that contains six transmembrane domains indicated as black bars. Beclin 1 binds to the N-terminus of IP_3R1 (suppressor domain), causing IP_3R sensitization that is required for driving mTOR-dependent autophagic flux. Bcl-2 inhibits IP_3R activity by binding with its N-terminal BH4 domain to a.a. 1389-1408 in the mouse IP_3R1 (green region of IP_3R1), located in the central, modulatory domain of the channel. Bok interacts with a.a. 1895-1903 (indicated as the red region) of the IP_3R. Through this interaction, Bok protects the channel from caspase 3-dependent cleavage by shielding a caspase 3-cleavage site (DEVD) that was identified at a.a. 1888-1892 in the mouse IP_3R1. Interaction of Bcl-2, Bcl-XL and Mcl-1 with the C-terminal region of the IP_3R (a.a. 2570-2749) appears to be dependent on the sixth transmembrane domain of the receptor, which results in sensitization of the channel to basal IP_3 levels. Phosphorylated K-Ras4B directly binds to the IP_3R (a.a. 2591-2734), and this binding is promoted in presence of Bcl-XL. Phosphorylated K-Ras4B counteracts the sensitization of IP_3R by Bcl-XL, thereby suppressing mitochondrial Ca^{2+} dynamics. In the absence of Bcl-XL, phosphorylated K-Ras4B mildly sensitizes the IP_3R to low [IP_3]. PKB/Akt suppresses IICR by phosphorylating S2681 in the C-terminal part of the IP_3R. In contrast, the Akt-mediated IP_3R inhibition is counteracted by PTEN, which dephosphorylates the IP_3R at S2681 via its protein phosphatase activity. Also, PML inhibits Akt-mediated phosphorylation of the IP_3R by recruiting PP2A to IP_3R-PKB/Akt-protein complexes at the MAMs, where PP2A then dephosphorylates active Akt. BRCA1 binds to the C-terminal tail of IP_3R (a.a. 2589-2749), thereby potentiating IP_3R-mediated Ca^{2+} signaling.

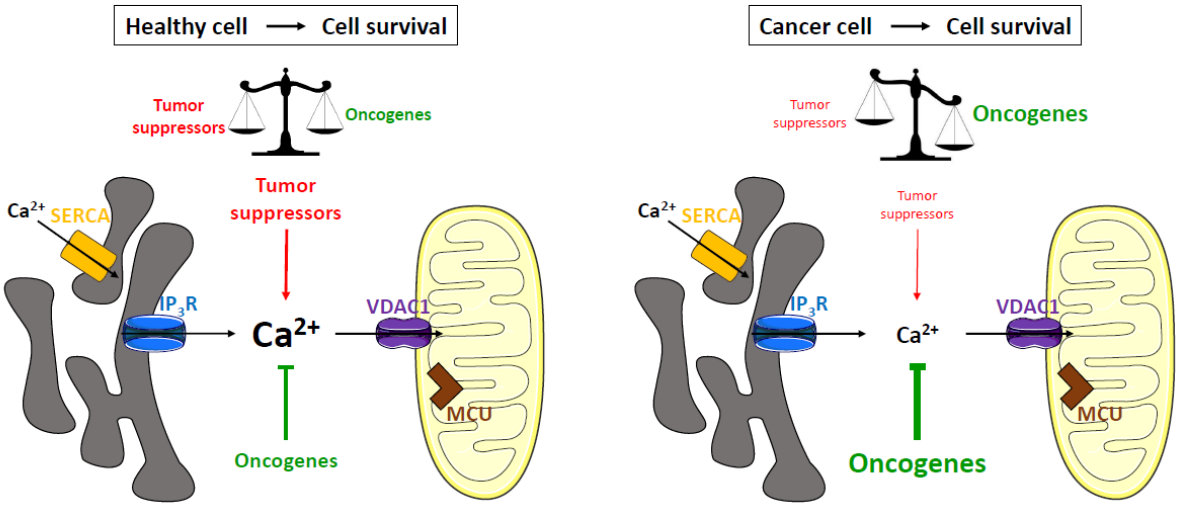

Figure 1

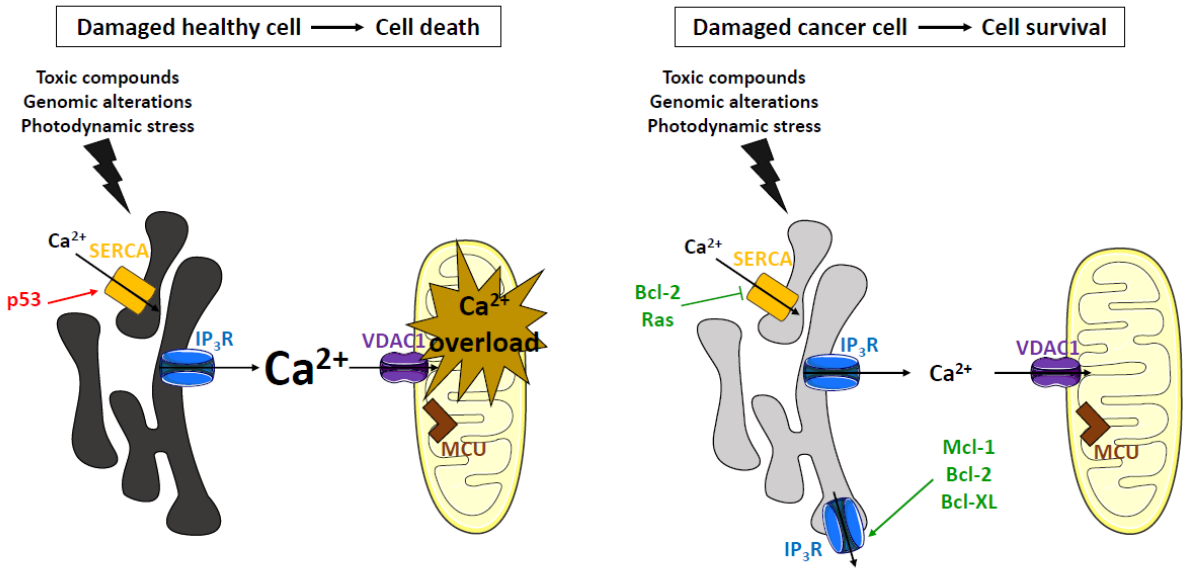

Figure 2

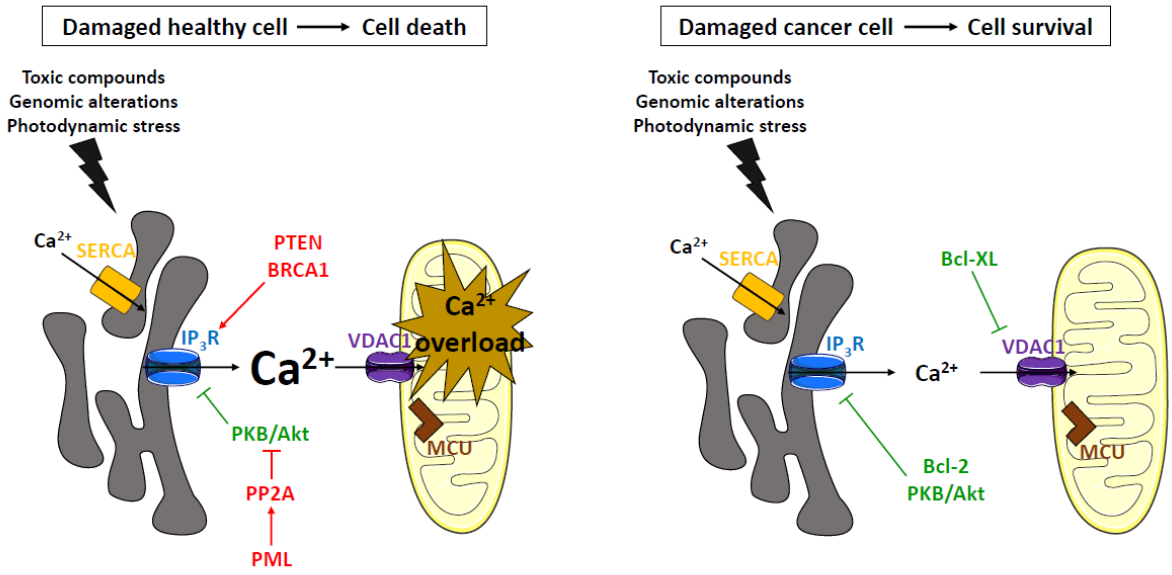

Figure 3

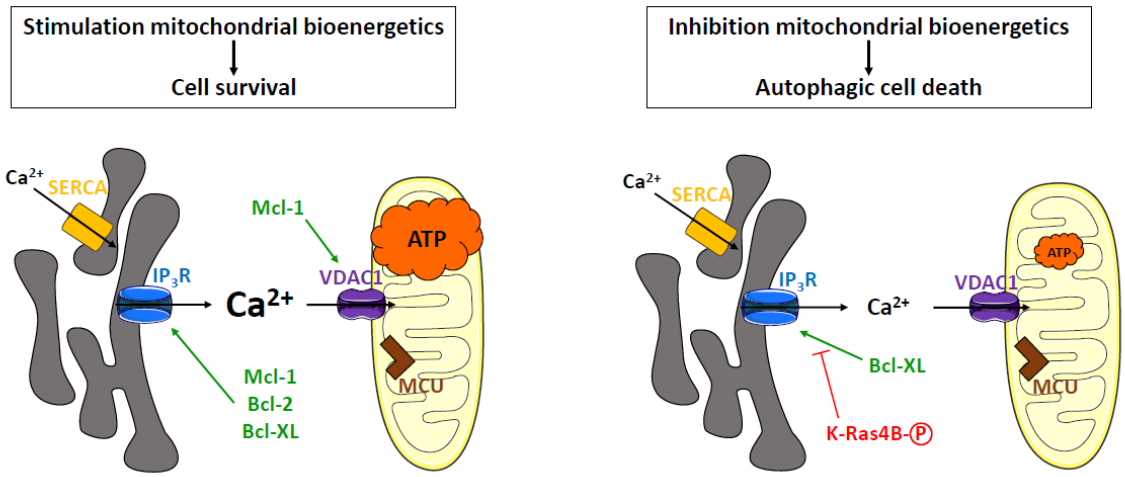

Figure 4

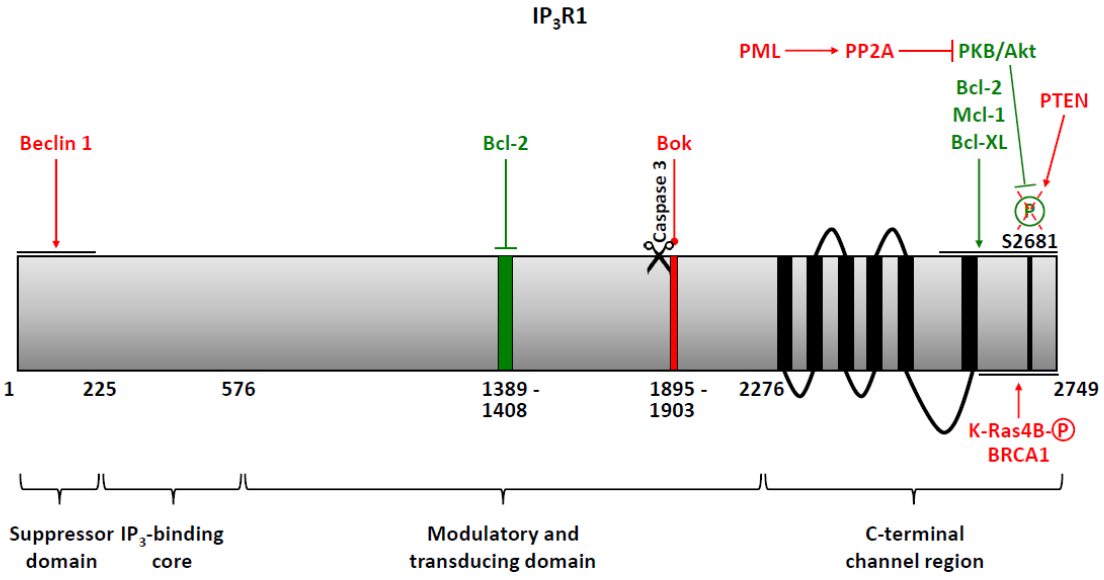

Figure 5

Highlights

- Altered intracellular Ca^{2+} signaling is a hallmark of cancer cells.
- Several oncogenes and tumor suppressors act at the endoplasmic reticulum, the main intracellular Ca^{2+} store.
- These proteins execute part of their functions through the modulation of intracellular Ca^{2+} homeostasis and dynamics.
- Dysregulation of oncogenes and tumor suppressors favors oncogenic Ca^{2+} signaling, supporting cell survival and tolerance to cell death.